THESIS

MICHIGAN STATE UNIVERSITY LIBRARIES

3 1293 01568 9817

This is to certify that the

dissertation entitled

Biology and Control of Hemp Dogbane
(Apocynum cannabinum L.)

presented by

Corey V. Ransom

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Crop and Soil Sciences

James J. Kelly
Major professor

Date April 12, 1997

MSU is an Affirmative Action/Equal Opportunity Institution

O-12771

LIBRARY
Michigan State
University

PLACE IN RETURN BOX to remove this checkout from your record.
TO AVOID FINES return on or before date due.

DATE DUE	DATE DUE	DATE DUE

MSU Is An Affirmative Action/Equal Opportunity Institution
c:\circ\datedue.pm3-p.1

BIOLOGY AND CONTROL OF HEMP DOGBANE (*Apocynum cannabinum* L.)

By

Corey V. Ransom

A DISSERTATION

Submitted to
Michigan State University
in partial fulfilment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Crop and Soil Sciences

1997

ABSTRACT

BIOLOGY AND CONTROL OF HEMP DOGBANE (*Apocynum cannabinum* L.)

By

Corey V. Ransom

Hemp dogbane is a perennial that is increasing as a weed problem in response to reduced tillage practices. It is difficult to control with herbicides and can reduce crop yields when growing unchecked. Hemp dogbane is morphologically variable and herbicidal control is often inconsistent. Studies were conducted to determine the level of genetic variation present among and within populations of hemp dogbane, and to identify effective postemergence herbicide strategies for control of hemp dogbane in corn. To examine variation among hemp dogbane populations, single plants of hemp dogbane were collected from populations in Michigan and Illinois. Variation among these collections was examined by observing their growth and morphology, and by genetic analysis using molecular techniques. A common garden experiment revealed that the collections of hemp dogbane differed greatly for numerous growth and morphological characteristics including stem height, shoot number, and shoot emergence pattern. Isozyme and RAPD analyses also revealed genetic variation among the hemp dogbane collections, although the level of variation was less than expected given the level of phenotypic differences observed among collections. To examine the level of variation within hemp dogbane populations, plants from two populations in Michigan were examined using isozyme and RAPD analyses. Plants from one of the populations tested were genetically identical suggesting the entire population was clonal. The other

population had some clonal individuals and some segregating individuals suggesting that outcrossing has occurred in that population. Control of hemp dogbane with postemergence herbicides in corn was examined at tilled and no-tillage sites for 3 years. Tank mixtures of ALS inhibitor and growth regulator herbicides provided greater and more consistent control than when single herbicides were applied alone. Postemergence control of hemp dogbane was highly dependent on the emergence patterns of the hemp dogbane, since shoots emerging after treatment were not controlled. Shoot emergence following herbicide application was less at the no-tillage sites than at the tilled sites in 2 of the 3 years of field research.

This dissertation is dedicated to my wife, Nancy and our children, Christopher and Rebecca and to everyone who has believed in me.

ACKNOWLEDGEMENTS

I would like to thank Dr. Jim Kells for his friendship, guidance and advice, and for the opportunity to pursue graduate work at Michigan State University. My thanks to my committee members, Dr. David Douches, Dr. Donald Penner, and Dr. Stephen Stephenson for their input and guidance in the development of this project. Special thanks to Dr. Douches for patience with a would-be geneticist, and to Dr. Penner for sharing his wealth of knowledge and experience.

I express gratitude to the technicians, graduate students, and student workers for their friendship and for help in conducting this research. They have been a great strength and their friendships are treasured.

Appreciation is expressed to the Corn Marketing Program of Michigan for their financial support of this research.

Thanks to the W. J. Beal Botanical Garden for *Apocynum* specimens used in this research.

Finally, thanks to my wife and children and our families for their support, love, and encouragement.

TABLE OF CONTENTS

TABLE OF CONTENTS (cont'd)

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

REVIEW OF LITERATURE

HEMP DOGBANE

1. Classification

Hemp dogbane (*Apocynum cannabinum* L.) is a member of the Apocynaceae. Taxonomic classification of hemp dogbane, and other species in *Apocynum* is difficult because of hybridization among the species (Anderson 1936; Balbach 1965; Voss 1996; Woodson 1930). Hemp dogbane has also been referred to as dogbane (Rickerl et. al 1994), common dogbane (Whitson 1991), and Indian-hemp (Hitchcock and Clothier 1898; Voss 1996).

2. Morphology

Hemp dogbane is an herbaceous perennial that reproduces by seed and creeping rootstock (Anonymous 1970). Hemp dogbane typically grows 30 to 60 cm in height (Anonymous 1970), but is capable of producing shoots in excess of 150 cm in height under ideal growth conditions (Whitson 1991). Plants grow erect with numerous ascending branches exceeding the main stem in height (Brown and Brown 1984). Stems are often red in color and contain a milky latex (Whitson 1991). Leaves are opposite or whorled (Whitson 1991), erect or ascending (Anonymous 1970), ovate to lanceolate, and 5 to 12 cm long with smooth margins (Anonymous 1970). Hemp dogbane leaves are glabrous to slightly pubescent with petioles 4 to 10 mm long to sessile (Anonymous 1970). Stomata appear to be limited to the lower surface of the leaves (Woodson 1930). Flowers have greenish or white petals 2 to 4 mm long, born in terminal cymes

(Anonymous 1970). The relative length of the corolla and the calyx are an important key in identifying the species of the *Apocynum.* In hemp dogbane the corolla is less than twice the length of the calyx (Woodson 1930). Seeds are born in narrow reddish brown follicles (Whitson 1991) 12 to 20 cm long that are slightly curved (Anonymous 1970). Seeds are 4 to 6 mm long, thin and flat with silky hairs attached to one end (Anonymous 1970).

3. Reproduction

Hemp dogbane can spread by seed and creeping rootstock (Woodson 1930).

Seed

Hemp dogbane plants average 20, and can produce up to 60 seed pods per plant (Evetts and Burnside 1972; Shultz and Burnside 1979b). Individual seed pods contain between 80 and 200 seeds (Evetts and Burnside 1972). Hemp dogbane seeds are susceptible to water stress and will not germinate at 9.1 bars of osmotic pressure, while germination is 91% at 0 bars (Evetts and Burnside 1972). Seeds from hemp dogbane have 74 to 99% germination the year they are produced, but germination rapidly declines after 1 year of burial in the soil (0 to 45%) and is essentially nonviable by the 3rd year of burial (Burnside et. al 1996; Burnside et al 1981; Evetts and Burnside 1972). Seed can germinate at temperatures between 10 and 40 C, and optimum seedling growth occurs at 20 to 25 C. Seedlings can emerge from a maximum of 4.3 cm in coarse textured soils and 3.3 cm in fine textured soils (Evetts and Burnside 1972). Seedlings are capable of regeneration following clipping 41 days after emergence (Robinson and Jeffery 1972).

Rootstock

Once established, hemp dogbane can spread by underground rootstock. After only a single growing season hemp dogbane roots were observed growing to a depth of 2.1 m and spread 3.6 m in length (Frazier 1944). By the second year, hemp dogbane roots penetrated to a depth of 4.2 m and spread radially 5.9 m (Frazier 1945). A single horizontal root of hemp dogbane has been excavated that was 8.8 m long (Hitchcock and Clothier 1898). New shoots can emerge both from crown buds and from buds forming on the creeping horizontal roots (Frazier 1944; Woodson 1930). Shoots appear to emerge in the spring in response to soil temperature. Shultz and Burnside (1979b) observed that shoots of hemp dogbane growing in a tilled nursery and in a sod waterway emerged when the soil temperature at the crown was 17 to 19 C. Similar to other perennial weeds, protein and carbohydrate levels in the roots are highest in the spring and fall and lowest during the summer months. Protein and non structural carbohydrates levels are highest in the lateral roots compared to the crown roots (Shultz and Burnside 1979b).

4. Distribution and Habitat

Hemp dogbane is native to the United States and can be found throughout most of North America (Balbach 1965; Becker 1981; Woodson 1930). Hemp dogbane can grow in diverse environments (Balbach 1965) and its growth habit is strongly affected by environmental conditions (Woodson 1930). Dense infestations of hemp dogbane are generally found in low-lying, wet areas, but are also present on drier upland soils (Becker 1981; Voss 1996). Studies have shown that soil type and fertility level affect hemp dogbane shoot height, branch length, leaf number, shoot weight, and root weight

(Robison and Jeffery 1972). Under dry conditions, hemp dogbane can form associations with vesicular arbuscular micorrhizal fungi, and the observed colonization level of hemp dogbane roots by the fungi directly correlate with phosphorous levels in the plant (Rickerl 1994).

5. Historical and Economic Uses

Native Americans used hemp dogbane as a fiber source for making bowstrings, nets (Hill 1952), rope, thread, and cloth (Woodson 1930). The latex in hemp dogbane was reportedly used as chewing gum by native Americans (Usher 1974). In recent history hemp dogbane has been examined as a source of latex for rubber production (Hill 1952), with plants containing greater than 5% latex (Woodson 1930). Hemp dogbane plants produce glycosides and alkaloids and have been investigated as a source for medicinal agents (Babcock 1962). Two of the glycosides produced by hemp dogbane, apocynin (Woodson 1930) and cymarin (Harris 1964), are cardiac glycosides with heart regulating properties similar to that of digitalin (Woodson 1930).

6. Frequency and Competitiveness in Crops

A survey of 60 irrigated corn fields in eastern Nebraska found that 40% had infestations of hemp dogbane (Evetts and Burnside 1973). Farmers participating in a survey in Ohio ranked hemp dogbane as the third most serious perennial broadleaf weed (Loux and Berry 1991). Another study conducted in Nebraska determined that of the fields infested with hemp dogbane, 60% had an average of less than 25 plants/ha, 35% had between 25 and 250 plants/ha, and 15% had greater than 250 plants/ha on an entire field basis (Schultz and Burnside 1979b). The average number of shoots within hemp dogbane patches ranged from 39,700 to 76,500 plants/ha. In a competition study, hemp dogbane was shown to reduce corn (*Zea mays* L.) yields by 8 to 10% and soybean (*Glycine max* L.) and sorghum (*Sorghum bicolor* L.) yields by as much as 41 and 45%, respectively (Shultz and Burnside 1979b). In the Nebraska study, hemp dogbane populations in irrigated fields averaged 25,000 plants/ha higher than those in non-irrigated fields. Hemp dogbane was present in all cropping systems, but was most prevalent in oats (*Avena sativa* L.) and soybeans, while least prevalent in alfalfa (*Medicago sativa* L.), pasture and wheat (*Triticum sativa* L.) (Shultz and Burnside 1979b). The author has observed hemp dogbane patches growing to the exclusion of crop plants suggesting that in well established hemp dogbane infestations, yield losses are probably higher than reported in the literature.

7. Response to Tillage

Hemp dogbane infestations increase as tillage decreases (Loux and Berry 1991; Triplett 1985; Triplett and Lytle 1972). Tillage may reduce the density of established hemp dogbane populations, but it may also increase the area infested by breaking the roots into small pieces and moving them throughout the tilled area (Robinson and Jeffery 1972; Shultz and Burnside 1979a; Woodson 1930). As early as 1930, Woodson (1930) recognized hemp dogbane as becoming a pest of agriculture through its spread by the tillage in cultivated fields. Hemp dogbane densities increased in corn as preplant tillage was decreased (Buhler et. al 1994). Hemp dogbane increases in reduced tillage systems because the underground roots are not disturbed and postemergence herbicide applications often do not correspond with the susceptible stage of hemp dogbane (Triplett

1985). In a long-term tillage study, Triplett and Lytle (1972) reported that hemp dogbane became a serious problem, invading numerous plots. Hemp dogbane did not invade tilled plots. Once established, herbicide treatments had little affect on hemp dogbane patches (Triplett and Lytle 1972). Buhler et. al (1994) observed that in a continuous corn rotation, hemp dogbane density was greater in no-tillage plots than in chisel or moldboard plowed plots. Crop rotation within the three tillage systems did not affect shoot densities of hemp dogbane.

8. Chemical Control

Hemp dogbane seedlings are generally controlled by herbicides used for annual broadleaf control. However, once established hemp dogbane control becomes more difficult (Triplett and Lytle 1972).

Shultz and Burnside (1970a) demonstrated that hemp dogbane emergence from crown and lateral roots could be suppressed or controlled up to 2 months following treatment using preemergence applications of thiocarbamate herbicides. However, control decreased by harvest.

Traditional postemergence herbicide recommendations for controlling hemp dogbane include glyphosate (*N*-phosphonomethyl)glycine), 2,4-D ((2,4-, 2,4-dichlorophenoxy) acetic acid), and dicamba (3,6-dichloro-2-methoxybenzoic acid). Glyphosate can effectively control hemp dogbane (Barnes and Brenchley 1972; Buhler et al 1994; Shultz and Burnside 1979b) and is most effective when applied in the fall (Barnes and Brenchley 1972; Shultz and Burnside 1979b). Barnes observed that glyphosate provided good control when applied to hemp dogbane in the late vegetative stage, but was ineffective when applied at the late flower stage (Barnes and Brenchley 1972). The addition of some surfactants to glyphosate increases control of hemp dogbane while the addition of other types of surfactants decrease hemp dogbane control with glyphosate (Wyrill and Burnside 1977). A weakness of glyphosate is that in the past it could only be applied in non-crop situations or in the fall following wheat harvest. More recently, glyphosate has become available for postemergence control of hemp dogbane in glyphosate resistant soybeans. Doll (1997) demonstrated that postemergence applications of glyphosate at rates higher than 0.84 kg/ha provided greater than 90% hemp dogbane control in glyphosate resistant soybeans.

Postemergence applications of 2,4-D in corn can provide good control of hemp dogbane (Glenn and Anderson 1993; Orfanedes and Wax 1991; Robinson and Jeffery 1972; Shultz and Burnside 1979a). Control is greatest when 2,4-D is applied at the late vegetative stage or in the fall as compared to spring applications (Orfanedes and Wax 1991; Robinson and Jeffery 1972). However, control with 2,4-D has been observed to be inconsistent from year to year (Glenn and Anderson 1993; Shultz and Burnside 1979a). Shultz and Burnside (1979a) also noted that farmers reported that hemp dogbane control with glyphosate and 2,4-D was erratic ranging from poor to excellent.

Dicamba is generally applied postemergence in corn at one half the rate of 2,4-D amine, and generally provides less control than 2,4-D (Glenn and Anderson 1993). Dicamba is also more effective when applied in the fall as opposed to spring (Robinson and Jeffery 1972). A comparison of hemp dogbane control with clopyralid (3,6-dichloro-2-pyridine carboxylic acid), fluoxypyr ([(4-amino-3,5-dichloro-6-flouro-2-pyridinyl)oxy]acetic acid), and 2,4-D showed that fluoxypyr was extremely active on hemp dogbane and when applied at 70 g/ha gave control similar to 560 g/ha of 2,4-D. Clopyralid provided little control of hemp dogbane regardless of rate or application timing (Orfanedes and Wax 1991). Additional studies were conducted to examine the differential response of hemp dogbane to two pyridine herbicides, fluoxypyr and clopyralid. Differences in absorption, translocation, and metabolism were observed between fluoxypyr and clopyralid, but did not account for their differential activity on hemp dogbane (Orfanedes et. al 1993).

Glenn and Anderson (1993) reported that tank mixtures containing nicosulfuron (2-[[[[(4,6-dimethoxy-pyrimidinyl)amino] carbonyl]amino]sulfonyl]-*N*,*N*-dimethyl-3-pyridine carboxamide) and 2,4-D or nicosulfuron and dicamba, provided 72 to 100% control of hemp dogbane.

Orfanedes and Wax (1991) observed resprouting of hemp dogbane plants near the base of controlled shoots 4 to 8 weeks after applications of fluoxypyr. Regrowth also appeared to be greater for treatments applied at the vegetative stage as compared to the early reproductive growth stage. Increased regrowth at the vegetative growth stage may be due to incomplete emergence of hemp dogbane shoots, or because translocation is acropetal during the vegetative growth preventing translocation of herbicide to the roots.

VARIATION IN WEEDS

1. Morphological Markers

Morphological and genetic variability has been observed in numerous weed species with perennial weeds comprising one half of the species reported as having biotypes or ecotypes. Perennial weeds with biotypes differing in growth and morphological characteristics include Canada thistle (*Cirsium arvense* (L.) Scop.) (Hodgson 1964), field bindweed (*Convolvulus arvensis* L.) (Degennaro and Weller 1984b), Quackgrass (*Elytrigia repens* (L.) Nevski) (Westra and Wyse 1981), Johnsongrass *(Sorghum halapense* (L.) Pers.) (McWhorter and Jordan 1976), yellow nutsedge *(Cyperus esculentus* L.) (Holt 1994), leafy spurge (*Euphorbia esula* L.) (Harvey et al. 1988), curly dock (*Rumex crispus* L.) (Hume and Cavers 1980), and salt cedar (*Tamarix ramoisissima* Ledeb.) (Wilkinson 1980). Differential response of biotypes to herbicides has been observed in field bindweed (DeGennaro and Weller 1984a), yellow nutsedge (Costa and Appleby 1976), and quackgrass (Alcantra et al. 1988). Variation among leafy spurge populations may affect the success of biological control agents introduced for its control (Harvey et al. 1988). Differences among weed populations can influence the competitive nature of the species and may affect its response to chemical or cultural control strategies. Morphological traits have limited use in population genetics studies because they are few in number, can be influenced by environmental conditions, and are often inherited by multiple genes (Nissen et. al 1995).

2. Molecular Markers

Recent advancements in molecular biology has provided additional techniques for analysis of genetic diversity among and within weed populations. These new techniques provide insights into weed biology and increase our knowledge in the area of weed population biology and genetics (Anonymous 1991). Molecular markers can be more powerful than morphological markers because they allow the detection of heterogeneity among plants that appear phenotypically identical (Anonymous 1991).

Isozyme Analysis

One technique that has been widely used to document genetic variation in weed species is isozyme analysis (Nissen et. al 1995). Isozymes are different molecular forms of the same enzyme or enzymes that have a common substrate but differ in their electrophoretic mobility (Wendel and Weeden 1989). Isozyme analysis is based on the electrophoretic separation of polymorphic protein isozymes in a starch gel (Anonymous 1991). Isozyme techniques are described in detail by Wendel and Weeden (1989). For isozyme analysis, fresh plant material is ground in a chilled extraction buffer designed to bind cellular phenolic compounds and prevent them from interfering with the protein extraction. The extract, including cellular proteins is absorbed into small filter paper wicks. The wicks are frozen and placed in a chilled starch gel and a charge is applied to the gel through a liquid buffer system. As charge is applied to the gel, the plant proteins move through the starch gel at different rates depending on their net charge. Once the enzymes have been separated on the gel, the gel is stained with enzyme specific staining solutions. The stains diffuse into the gel and react with regions where specific enzymes are present, resulting in the precipitation of soluble dye indicators in regions of enzyme activity. Colored banding patterns then become apparent, and differences in these patterns suggest different forms of the enzyme being analyzed. Isozyme analysis is genetically based since enzyme proteins are coded for by the plant DNA (Wendel and Weeden 1989). Isozymes are a popular tool for genetic analysis because they are relatively simple and can be used to generate a series of scoreable single gene markers (Wendel and Weeden 1989).

Some weaknesses of isozyme analysis are that a limited number of loci can be sampled and allozyme variation may be too low for analysis of genetic diversity within and among plant populations (Nissen et. al 1995). Isozyme analysis has been used to document genetic variation among and within populations of yellow nutsedge (Horak and Holt 1986; Horak et. al 1987), broom snakeweed (*Gutierrezia sarothrae* (Pursh) Britt. & Rusby) (Hou and Sterling 1995), purple loosestrife (*Lythrum salicaria* L.) (Strefeler et. al 1996), common lambsquarters (*Chenopodium album* L.) (Mouemar and Gasquez 1983), velvetleaf (*Abutilon theophrasti* Medicus), jimsonweed (*Datura stramonium* L.), wild proso millet (*Panicum miliaceum* L.), johnsongrass, and giant foxtail (*Setaria faberi* Herrm.) (Warwick 1990). Isozyme analysis has also been employed for the study of pollen flow in common milkweed (*Asclepias syriaca* L.) (Shore 1993). Considerable information about the genetic relationships among *Conyza* species (Thébaud and Abbott 1995) and among the *Setaria* species (Wang et. al 1995a, 1995b) and within the *Daucus carota* L. (St. Pierre et. al 1990) and *Carex pachystachya* Cham. (Whitkus 1992) complexes have been generated utilizing isozyme analysis.

RAPD Analysis

Randomly Amplified Polymorphic DNA (RAPD) analysis is based on the Polymerase Chain Reaction (PCR) and involves the amplification of small sequences of target DNA using random primers. As a DNA-based marker, the strength of RAPD analysis is the ability to assess genetic variation throughout the genome and generate nearly limitless numbers of characters for evaluation (Nissen et. al 1995). This makes RAPD analysis a powerful tool for assessing genetic variation within and among weed populations.

RAPD analysis is described by Nissen et. al (1995). For RAPD analysis, DNA is extracted from the plants of interest and combined in a small reaction tube with all the components needed for DNA replication to occur. The reactants include the plant DNA of interest, a thermostable polymerase, nucleotides, and random primers. The reaction tube is place in a thermocycler and the temperature is cycled through various temperatures causing a series of processes to take place in the tube. A high temperature (92 to 98 C) is used to separate DNA into single strands, a low temperature (37 to 50 C) is used to allow the random primers in the reaction to bind to complementary bases on the plant DNA, and a medium temperature (72 C) allows DNA replication to take place. This temperature regime is repeated many times (30 to 40) resulting in the amplification of specific DNA sequences found in the target DNA. DNA amplified by RAPD analysis must be flanked by sequences complementary to the random primers and be less than 4000 base pairs in length (Nissen et. al 1995). The amplified DNA products from the RAPD analysis are then separated electrophoretically on an agarose gel and the gel is stained with ethidium bromide (EtdBr) and bands of amplified DNA are visualized as they fluoresce under ultraviolet light. Bands are dominant and are scored among plants as either present or absent. The absence of a particular size band may be due to a deletion, insertion or other mutation that prevents a particular sequence from being amplified or results in a different size sequence being amplified (Nissen et. al 1995). The detection of sequences amplified in one population and not another can then be used as a DNA marker (Nissen et. al 1995). RAPD analysis has been used to analyze different populations of leafy spurge and may be an important tool for determining the susceptibility of leafy spurge populations to biological control agents (Nissen et. al 1995). RAPD analysis has also been used to look at relationships among populations of cultivated and weedy eggplant (*Solanum melongena* L.) species (Karihaloo et. al 1995) and to examine genetic variation among and within populations of wild mustard (*Sinapsis arvensis* L.) (Moodies et. al 1997). RAPD analysis provides a powerful tool for studying all aspects of weed taxonomy and population genetics.

LITERATURE CITED

LITERATURE CITED

Alcantara, E. N., D. L. Wyse, and J. M. Spitzmueller. 1989. Quackgrass (*Agropyron repens*) biotype response to sethoxydim and haloxyfop. Weed Sci. 37: 107-111.

Anderson, E. 1936. An experimental study of hybridization on the genus *Apocynum*. Ann. Missouri Bot. Gard. 23:159-169.

Anonymous. 1991. An overview of new techniques and advances in weed physiology and molecular biology. Weed Sci. 39:480-481.

Anonymous. 1981. Weeds of the North Central States. North Central Region Research Publication No. 281, Bulletin 772. University of Illinois at Urbana-Champaign. p.134.

Anonymous. 1970. Selected Weeds of the United States. Agricultural handbook No. 336. Agricultural Research Service. United States Department of Agriculture. Pages 284-285.

Babcock, Philip A., and D. P. Carew. 1962. Tissue culture of the Apocynaceae. I. Culture requirements and alkaloid analysis. Lloydia 25:209-213.

Balbach, H. E. 1965. Variation and speciation in populations of Apocynaceae in North America. Ph.D. Thesis. Univ. Illinois at Urbana Champaign, Urbana, Ill.

Barnes, D. and R. Brenchley. 1972. Response of hemp dogbane to the isopropylamine salt of ghyphosate (MON-2139). Proceedings Central Weed Control Conf. 27:54-55.

Becker, R. 1981. Todays Weed: hemp dogbane. Weeds Today. Winter Issue. pp. 15-16.

Buhler, D.B., D.E. Stoltenberg, R. L. Becker, and J. L. Gunsolus. 1994. Perennial Weed Populations after 14 years of variable tillage and cropping practices. Weed Sci. 42:205-209.

Burnside, O. C., R. G. Wilson, S. Weisberg, and K. G. Hubbard. 1996. Seed longevity of 41 weed species buried 17 years in eastern and western Nebraska. Weed Sci. 44:74-86

Burnside, O. C., C. R. Fenster, L. L. Evetts, and R. F. Mumm. 1981. Germination of exhumed weed seed in Nebraska. Weed Sci. 29:577-586

Costa, J. and A. P. Appleby. 1976. Response of two yellow nutsedge varieties to three herbicides. Weed Sci. 24: 54-58.

Degennaro, F. P. and S. C. Weller. 1984. Differential susceptibility of field bindweed (*Convolvulus arvensis*) biotypes to glyphosate. Weed Sci. 32: 472-476.

Degennaro, F. P. and S. C. Weller. 1984. Growth and reproductive characteristics of field bindweed (*Convolvulus arvensis*) biotypes. Weed Sci. 32: 525-528.

Doll, J. D. 1997. Hemp dogbane (Apocynum cannabinum L.) management in corn and gyphosate-resistant soybeans. Abstr. Weed Sci. Soc. Am. 37:90.

Dyer, W. E. 1991. Applications of molecular biology in weed science. Weed Sci. 39:482-488.

Evetts, L. C. and O. C. Burnside. 1972. Germination and seedling development of common milkweed and other species. Weed Sci. 20:371-378.

Evetts, L. C. and O. C. Burnside. 1973. Watch out for hemp dogbane. Nebraska Farm, Ranch, and Home Quart. 19:19-20.

Frazier, J. C. 1944. Nature and rate of development of root systems of *Apocynum cannabinum*. Bot. Gaz. 105:463-470.

Frazier, J. C. 1945. Second year development of root systems of *Apocynum cannabinum*. Bot. Gaz. 106:332.

Glenn, S. and N. G. Anderson. 1993. Hemp dogbane (*Apocynum cannabinum*) and wild blackberry (*Rubus allegheniensis*) control in no-tillage corn (*Zea mays*). Weed technol. 7:47-51.

Harris, A. L., H. B. Nylund, and D. P. Carew. 1964. Tissue culture studies of certain members of the Apocynaceae. Lloydia. 27: 322-327.

Harvey, S. J., R. M. Nowierski, P. G. Mahlberg, and J. M. Story. 1988. Taxonomic evaluation of leaf and latex variability of leafy spurge (*Euphorbia* spp.) for Montana and European accessions. Weed Sci. 36: 726-733.

Hill, A. F. 1952. Page 34 *in* Economic botany. Second edition. Maple Press Co., York, PA. p. 34.

Hitchcock, A. S. and G. L. Clothier. 1898. Vegetative Propagation of perennial weeds. Fifth Report on Kansas Weeds. Bull. 78: 1-22.

Hodgson, J. M. 1964. Variations in ecotypes of Canada thistle. Weeds 12:167-171.

Holt, J. S. 1994. Genetic variation in life history traits in yellow nutsedge (*Cyperus esculentus*) from California. Weed Sci. 42: 378-384.

Horak, M. J., J. S. Holt, and N. C. Ellstrand. 1987. Genetic variation in yellow nutsedge (*Cyperus esculentus*). Weed Sci 35:506-512.

Horak, M. J., and J. S. Holt. 1986. Isozyme variability and breeding systems in populations of yellow nutsedge (*Cyperus esculentus*). Weed Sci 34:538-543.

Hume, L. and P. B. Cavers. 1982. Geographic variation in the widespread perennial weed, *Rumex crispus*. The relative amounts of genetic and environmentally induced variation among populations. Can. J. Bot. 60:1928-1937.

Karihaloo, J. L., S. Brauner, and L. D. Gottlieb. 1995. Random amplified polymorphic DNA variation in the eggplant, *Solanum melongena* L. (Solanaceae). Theor Appl. Genet. 90:767-770.

Kiltz, B. F. 1929. Perennial weeds which spread vegetatively. J. Am. So. Agron. 22: 217-234.

Klingaman, T. E. and L. R. Oliver. 1996. Existence of ecotypes among populations of entireleaf morningglory (*Ipomea hederacea* var. *integriuscula*). Weed Sci. 44:540-544.

Kropp, B. K., S. Albee, K. M. Flint, P. Zambino, L. S. Szabo, and S. V. Thomson. 1995. Early detection of systemic rust infections of dyers woad (*Isatis tinctoria*) using the polymerase chain reaction. Weed Sci. 43:467-472.

Loux M. M. and M. A. Berry. 1991. Use of a grower survey for estimating weed problems. Weed Technol. 5:460-66.

McWhorter, C. G. and T. N. Jordan. 1976. Comparative morphological development of six johnsongrass ecotypes. Weed Sci. 24:270-275.

Moodies, M., R. P. Finch, and G. Marshall. 1997. Analysis of genetic variation in wild mustard (*Sinapsis arvensis*) using molecular markers. Weed Sci. 45:102-107.

Mouemar, A. A. and J. Gasquez. 1983. Environmental conditions and isozyme polymorphism in *Chenopodium album* L. Weed Res. 23:141-149.

Nissen, S. J., R. A. Masters, D. J. Lee, and M. L. Rowe. 1995. DNA-based marker systems to determine genetic diversity of weedy species and their application to biocontrol. Weed Sci. 43:504-513.

Orfanedes, M.S. and L.M. Wax. 1991. Differential response of hemp dogbane (*Apocynum cannabinum*) to clopyralid, dowco 433, and 2,4-D. Weed Technol. 5:782-788.

Rickerl, D. H., F. O. Sancho, and S. Ananth. 1994. Vesicular arbuscular endomycorrhizal colonization of wetland plants. J. Environ. Qual. 23:913-916.

Robinson, L. R. and L. S. Jeffery. 1972. Hemp dogbane growth and control. Weed Sci. 20:156-159.

Schultz, M. E. and O. C. Burnside. 1979a. Control of hemp dogbane with foliar and soil applied herbicides. Agron. J. 71:723-729.

Schultz, M. E. and O. C. Burnside. 1979b. Distribution, competition, and phenology of hemp dogbane (*Apocynum cannabinum*) in Nebraska. Weed Sci. 27:565-570.

Shore, J. S. 1993. Pollination genetics of common milkweed, *Asclepias syriaca* L. Hered. 70:101-108.

St. Pierre, M. D., R. J. Bayer, and I. M. Weis. 1990. An isozyme-based assessment of the genetic variability within the Daucus carota complex (Apiaceae: Caucalidae). Can. J. Bot. 68:24449-2457.

Strefeler, M. S., E. Darmo, R. L. Becker, and E. J. Katovich. 1996. Isozyme characterization of genetic diversity in Minnesota populations of purple loosestrife, *Lythrum salicaria* (Lythraceae). Amer. J. Bot. 83:265-273.

Thébaud, C. and R. J. Abbott. 1995. Characterization of invasive *Conyza* species (Asteraceae) in Europe: Quantitative trait and isozyme analysis. Amer. J. Bot. 82:360-368.

Triplett, G. B. Jr. 1985. Principles of weed control for reduced-tillage corn production. Pages 26-40 *in* A. F. Weise, ed. Weed control in limited tillage systems. Weed Sci. Soc. Am. Mongr. 2, Champaign, IL.

Triplett, G. B. Jr. and G. D. Lytle. 1972. Control and ecology of weeds in continuous corn grown without tillage. Weed Sci. 20:453-457.

Usher, G. 1974. Page 52 *in* A dictionary of plants used by man. Constable and Co., London. 619 pp.

Voss, E. G. 1996. Michigan flora: Part III. Dicots (Pyrolaceae-Compositae). Cranbrook Institute of Science Bulletin 61 and University of Michigan Herbarium. pp. 84-87.

Wang, R. L., J. F. Wendel, and J. H. Dekker. 1995. Weedy adaptation in *Setaria* spp. I. Isozyme analysis of genetic diversity and population genetic structure in *Setaria viridis*. Amer. J. Bot. 82:308-317.

Wang, R. L., J. F. Wendel, and J. H. Dekker. 1995. Weedy adaptation in *Setaria* spp. II. Genetic diversity and population genetic structure in *S. glauca*, *S. geniulata*, and *S. faberii* (Poaceae). Amer. J. Bot. 82:1031-1039.

Warwick, S. I. 1990. Allozyme and life history variaiton in five northwardly colonizing North American weed species. Plant Syst. Evol. 169: 41-54.

Wendel, J. F. and N. F. Weeden. 1989. Visualization and interpetation of plant isozymes. Pages 4-45 *in* D. E. Soltis and P. S. Soltis eds., Isozymes in plant biology. Dioscorides Press, Portland, OR. 4-45 pp.

Westra, P. H. and D. L. Wyse. 1981. Growth and development of quackgrass (*Agropyron repens*) biotypes. Weed Sci. 29: 44-52.

Whitkus, R. 1992. Allozyme variation within the *Carex pachystachya* complex (Cyperaceae). Syst. Bot. 17:16-24.

Whitson, T. D. ed. 1991. Weeds of the West. West. Soc. Weed Sci., Wyoming. 30-31 pp.

Wilkinson, R. E. 1980. Ecotypic variation of *Tamarix pentandra* epicuticular wax and possible relationship with herbicide sensitivity. Weed Sci. 29: 110-113.

Woodson, R. E. Jr. 1930. Studies in the Apocynaceae I. Ann. Missouri Bot. Gard. 17:1-212.

CHAPTER 2

MORPHOLOGICAL VARIATION AMONG HEMP DOGBANE (*Apocynum cannabinum* L.) POPULATIONS

ABSTRACT

Studies were conducted at East Lansing, Michigan and Champaign, Illinois to study morphological variation among hemp dogbane populations. Plants were collected from locations throughout Michigan and Illinois. Eight collections from each state were used to establish 16 collections at nurseries in East Lansing, Michigan and Champaign, Illinois. Growth, stem characteristics, and leaf characteristics were measured for each collection at both nurseries. Differences among collections were observed for all measurements with the exception of emergence date and growing degree to emergence. The number of shoots produced by the collections ranged from 5 to 54, while shoot height ranged from 69 to 126 centimeters. Collections spread laterally at different rates with the most aggressive collection covering 19 times more plot area than the least aggressive collection. Total shoot dry weight varied greatly among collections. Some collections could be distinguished from others by their unique leaf shape. Shoot number, shoot height, and plot area were greater for collections growing in East Lansing. Differences in growth and morphological characteristics among collections were not correlated with the location from which the collections originated. Differences in measurements between the two nurseries illustrates the role of environment and genetics in the growth and morphology of this plant species. **Nomenclature:** hemp dogbane,

Apocynum cannabinum L. #[1] APPCA.

Additional index words. Morphology, variation, population, weed ecology, diversity, biotype, ecotype, *Apocynum cannabinum*.

INTRODUCTION

Hemp dogbane is a perennial weed that is native to the United States and can be found throughout most of North America (Balbach 1965; Becker 1981; Woodson 1930). Hemp dogbane is common to field crops and has been shown to reduce corn yields 8 to 10% and soybean and sorghum yields by as much as 41 and 45%, respectively (Shultz and Burnside 1979a). With increases in reduced tillage acreage, hemp dogbane is becoming a greater problem in field crops because infestations increase as tillage decreases (Loux and Berry 1991; Triplett 1985; Triplett and Lytle 1972). In addition, chemical control of hemp dogbane is difficult and often inconsistent (Glenn and Anderson 1993; Shultz and Burnside 1979b).

Hemp dogbane reproduces by seed and creeping rootstock (Woodson 1930). Hemp dogbane plants can produce thousands of seeds, but seed germination declines rapidly over time (Burnside et al. 1996; Shultz and Burnside 1979a). Hemp dogbane typically grows 30 to 60 cm in height, but is capable of producing shoots in excess of 150 cm in height under ideal growth conditions (Woodson 1930). It can grow in diverse

---

[1]Letters following this symbol are WSSA-approved computer code from Composite List of Weeds, Weed Sci. 32, Suppl. 2. Available from WSSA, 1508 W. University Ave., Champaign, IL 61821-3133.

environments (Balbach 1965) and its growth habit is strongly affected by environmental conditions (Woodson 1930). Studies have shown that soil type and fertility level affect hemp dogbane shoot height, branch length, leaf number, shoot weight, and root weight (Robison and Jeffery 1972). Hybridization occurs between hemp dogbane and other species in the *Apocynum* making taxanomic classification difficult and increasing the genetic variability within each species (Anderson 1936; Balbach 1965; Woodson 1930).

Morphological and genetic variability has been observed in numerous weed species with perennial weeds comprising half of the species reported as having biotypes or ecotypes. Perennial weeds with biotypes differing in growth and morphological characteristics include Canada thistle (Hodgson 1964), field bindweed (Degennaro and Weller 1984b), quackgrass (Westra and Wyse 1981), johnsongrass (McWhorter and Jordan 1976), yellow nutsedge (Holt 1994), leafy spurge (Harvey et al. 1988), curly dock (Hume and Cavers 1980), and salt cedar (Wilkinson 1980). Differential response of biotypes to herbicides has been observed in field bindweed (DeGennaro and Weller 1984a), yellow nutsedge (Costa and Appleby 1976), and quackgrass (Alcantra et al. 1988). Variation among leafy spurge populations may affect the success of biological control agents intoduced for its control (Harvey et al. 1988). Differences between weed populations can influence the competitive nature of a weed species and may affect its response to chemical or cultural control strategies. Most biotype studies previously reported have been conducted under a single environment. It is beneficial to study biotype growth responses under at least two environments to determine how plant genetics and environmental conditions influence plant morphology.

The objectives of this study were to a) determine if geographically isolated populations of hemp dogbane exhibit morphological variation when grown in a common environment, and b) determine the affect of location on hemp dogbane population growth and morphology.

MATERIALS AND METHODS

During September and October of 1989, hemp dogbane plants were collected from 27 locations across Michigan and 23 locations across Illinois. At each location a single rootstock was collected so that resulting propagules would be genetically identical. Rootstocks were divided and planted into pots in the greenhouse. Plants were maintained and observed in the greenhouse until the spring of 1990. Based on differences observed in the greenhouse, plants from eight Michigan locations and eight Illinois locations were selected for evaluation. Plants from all 16 locations were used to establish nurseries in both East Lansing, MI and Champaign, IL during spring of 1990. The original locations of each collection are presented in Table 1. To establish the nurseries, plants from the greenhouse were planted in the center of 1.2 by 1.2 m plots with 1.5 m buffer strips on all sides. Plants were watered weekly for the first month with rainfall only for the remainder of the experiment. Plants were allowed to establish and grow free from competition with other weeds. Leaf and shoot characteristics including shoot number, shoot height, stem color, growth habit, petiole length, leaf length, width, and leaf pubescence were measured during July, August, and September 1990. The evaluations were repeated in 1991 during June, July, August, and September. Shoot and leaf data presented in this paper are from evaluations taken August 1991. Additional measurements were taken in 1991 including emergence date, growing degree day to emergence, plot area, senescence date, growing degree days to 50% leaf drop, and total shoot dry weight. Plot area was determined in September, 1991 and total shoot dry weight was determined in November, 1991 by harvesting all of the shoots in the plot. Shoot height was evaluated by measuring the tallest shoot in each plot. Leaf length and width and petiole length were taken from five leaves at mid canopy and values were averaged. Growing degree days were calculate using a 50 F base beginning March 1, 1991. Stem color, upper and lower leaf pubescence, and growth habit were evaluated visually. Emergence and senescence dates were calculated as days beginning March 1, 1991.

Plots were arranged in a completely randomized block design replicated four times. Measurements were analyzed using ANOVA and means were separated using Fisher's LSD test at the 5% level. Where location by collection interactions were present, data from each location were analyzed separately. Data were combined for analysis when interactions were not present.

RESULTS AND DISCUSSION

Location means for hemp dogbane measurements are listed in Table 2. Hemp dogbane emergence date was not affected by location, while growing degree day to emergence was greater at the Champaign location because of its more southern latitude. Growing degree days to 50% leaf drop was also greater at the Champaign nursery likely due to the longer growing season in Illinois. Senescence date was influenced by location and collection.

Collections growing in East Lansing produced more shoots and shoots were taller when compared with the collections growing in Champaign. Plot area was twice as large for collections growing in East Lansing as compared to those growing in Champaign. Mean shoot dry weights were greatest at Champaign although there were significant location by collection interactions. Petioles in general were longer at Champaign, but had a significant location by collection interaction. Leaf width and length were not affected by location, but the leaf length to width ratio was significantly different, with collections at the East Lansing nursery producing longer leaves in proportion to their width than those growing in Champaign.

Differences were evident among collections for all measurements except growing degree days to emergence. Collection means for hemp dogbane growth characteristics are found in Table 3. Initiation of shoot emergence differed by 18 days among collections. Growing degree days to emergence was variable ranging from 116 to 364. Growing degree days to 50% leaf drop was significant among the collections with the highest and lowest collections differing by 224 growing degree days. Senescence dates were different among collections growing at each location.

In addition to differences in initiation of shoot emergence, collections also differed in the relative time over which shoots emerged with some collections producing more than 80% of their shoots in June and others not producing greater than 80% of their shoots until August. Cumulative shoot numbers presented as the percent of the maximum number of shoots produced were significant among collections in June and July (Table 4).

Dramatic differences in shoot characteristics and plot area (Table 5) were apparent among collections. The number of shoots produced by the collections ranged from 5 to 54, while shoot height ranged from 69 to 126 centimeters. All but two collections produced greater shoot dry weight when growing at the Champaign nursery, and in general plants producing few shoots had the lowest shoot dry weight. The same collection produced the lowest shoot dry weight at both locations, and high and low shoot dry weight differed by 380 and 769 grams at East Lansing and Champaign, respectively. Collections spread laterally at different rates with the most aggressive collection covering 19 times more plot area than the least aggressive collection. The number of shoots produced was highly correlated with the plot area of each collection. Distinctive growth characteristics made visual identification of some collections possible. Certain collections produced numerous tall shoots and spread widely throughout the study area, while others produced only a few shorter shoots and spread very little. Although emergence date and shoot dry weight were weakly correlated, some of the earlier emerging collections produced the greatest shoot biomass and some of the later emerging collections produced the least shoot biomass.

Differences in petiole length and leaf characteristics (Table 6) were also observed among hemp dogbane collections. While there was a collection by location interaction, the collection with the longest petioles and the collection with the shortest petioles were the same at both locations. Hemp dogbane leaves ranged from 50 to 72 mm long and 18 to 29 mm wide. Differences among collections for the leaf length to width ratio were readily apparent and some collections could be identified from others by their unique leaf shape. The upper leaf surfaces of all collections observed were glabrous, while pubescence was present on the lower leaf surface of some collections. Stem color was consistently red for some collections, while varying from reddish green to red in others. Leaf pubescence and stem color appear to be influenced strongly by environmental conditions and while consistent for some collections, were highly variable in others.

Variability observed among hemp dogbane populations is not surprising since a high level of genetic heterogeneity is expected in plant species that are able to grow in a wide range of environmental conditions (Hume and Cavers 1982). The range between the highest and lowest dry shoot weights of hemp dogbane populations was much greater than reported among quackgrass (Westra and Wyse 1981) and yellow nutsedge (Holt 1994) biotypes. Research on ivyleaf morningglory ecotypes has shown that ecotype flowering dates were strongly correlated with the latitude the plants originated from (Klingaman and Oliver 1996). No correlation was observed between the morphological and growth characteristics evaluated in this study and the geographic region where the plants were collected. Collections separated by larger geographic distances would be expected to be more divergent than those from the same location. This research suggests that variability among populations of hemp dogbane is affected more by local environmental differences than by overall geographic distance. Considerable variation was apparent among populations of hemp dogbane growing at each nursery. In addition, the influence of environment on plant morphology and growth was apparent. Differences would be expected between hemp dogbane plants growing under the different environmental conditions at each nursery since hemp dogbane growth is influenced by soil type and fertility level (Robison and Jeffery 1972). Changes in growth patterns has also been reported in quackgrass biotypes grown at different locations (Westra and Wyse 1981). Morphological variation present among populations of hemp dogbane may influence the competitiveness of a population in a cropping situation and may determine its response to mechanical and chemical control. Weed science research needs to account for species variation to avoid making invalid assumptions about weed species responses.

LITERATURE CITED

LITERATURE CITED

Alcantara, E. N., D. L. Wyse, and J. M. Spitzmueller. 1989. Quackgrass (*Agropyron repens*) biotype response to sethoxydim and haloxyfop. Weed Sci. 37: 107-111.

Anderson, E. 1936. An experimental study of hybridization on the genus *Apocynum*. Ann. Missouri Bot. Gard. 23:159-169.

Balbach, H. E. 1965. Variation and speciation in populations of Apocynaceae in North America. Ph.D. Thesis. Univ. Illinois at Urbana Champaign, Urbana, Ill.

Becker, R. 1981. Todays Weed: hemp dogbane. Weeds Today. Winter Issue. pp. 15-16.

Burnside, O. C., R. G. Wilson, S. Weisberg, and K. G. Hubbard. 1996. Seed longevity of 41 weed species buried 17 years in eastern and western Nebraska. Weed Sci. 44:74-86

Costa, J. and A. P. Appleby. 1976. Response of two yellow nutsedge varieties to three herbicides. Weed Sci. 24: 54-58.

Degennaro, F. P. and S. C. Weller. 1984a. Differential susceptibility of field bindweed (*Convolvulus arvensis*) biotypes to glyphosate. Weed Sci. 32: 472-476.

Degennaro, F. P. and S. C. Weller. 1984b. Growth and reproductive characteristics of field bindweed (*Convolvulus arvensis*) biotypes. Weed Sci. 32: 525-528.

Glenn, S. and N. G. Anderson. 1993. Hemp dogbane (*Apocynum cannabinum*) and wild blackberry (*Rubus allegheniensis*) control in no-tillage corn (*Zea mays*). Weed Technol. 7:47-51.

Harvey, S. J., R. M. Nowierski, P. G. Mahlberg, and J. M. Story. 1988. Taxonomic evaluation of leaf and latex variability of leafy spurge (*Euphorbia* spp.) for Montana and European accessions. Weed Sci. 36: 726-733.

Hodgson, J. M. 1964. Variations in ecotypes of Canada thistle. Weeds 12:167-171.

Holt, J. S. 1994. Genetic variation in life history traits in yellow nutsedge (*Cyperus esculentus*) from California. Weed Sci. 42: 378-384.

Hume, L. and P. B. Cavers. 1982. Geographic variation in the widespread perennial weed, *Rumex crispus*. The relative amounts of genetic and environmentally induced variation among populations. Can. J. Bot. 60:1928-1937.

Klingaman, T. E. and L. R. Oliver. 1996. Existence of ecotypes among populations of entireleaf morningglory (*Ipomea hederacea* var. *integriuscula*). Weed Sci. 44:540-544.

Loux M. M. and M. A. Berry. 1991. Use of a grower survey for estimating weed problems. Weed Technol. 5:460-66.

McWhorter, C. G. and T. N. Jordan. 1976. Comparative morphological development of six johnsongrass ecotypes. Weed Sci. 24:270-275.

Robison, L. R. and L. S. Jeffery. 1972. Hemp dogbane growth and control. Weed Sci. 20:156-159.

Schultz, M. E. and O. C. Burnside. 1979a. Distribution, competition, and phenology of hemp dogbane (*Apocynum cannabinum*) in Nebraska. Weed Sci. 27:565-570.

Schultz, M. E. and O. C. Burnside. 1979b. Control of hemp dogbane with foliar and soil applied herbicides. Agron. J. 71:723-729.

Triplett, G. B. Jr. 1985. Principles of weed control for reduced-tillage corn production. Pages 26-40 in Weed control in limited tillage systems, A. F. Weise, ed. Weed Sci. Soc. Am. Mongr. 2, Champaign, IL.

Triplett, G. B. Jr. and G. D. Lytle. 1972. Control and ecology of weeds in continuous corn grown without tillage. Weed Sci. 20:453-457.

Westra, P. H. and D. L. Wyse. 1981. Growth and development of quackgrass (*Agropyron repens*) biotypes. Weed Sci. 29: 44-52.

Wilkinson, R. E. 1980. Ecotypic variation of *Tamarix pentandra* epicuticular wax and possible relationship with herbicide sensitivity. Weed Sci. 29: 110-113.

Woodson, R. E. Jr. 1930. Studies in the Apocynaceae I. Ann. Missouri Bot. Gard. 17:1-212.

Table 1. County and state of origin of hemp dogbane collections.

	Collection Area	
Collection	County	State
M-101	Shiawassee	Michigan
M-102	Huron	Michigan
M-105	Arenac	Michigan
M-108	Cass	Michigan
M-109	Monroe	Michigan
M-112	Kalamazoo	Michigan
M-118	Allegan	Michigan
M-124	Tuscola	Michigan
I-102	Jackson	Illinois
I-103	Adams	Illinois
I-107	Woodford	Illinois
I-109	Iroquois	Illinois
I-115	LaSalle	Illinois
I-116	LaSalle	Illinois
I-122	Livingston	Illinois
I-124	Randolph	Illinois

Table 2. Location means for hemp dogbane measurements.

	Location		Significance[a]	
Measurement	Michigan	Illinois	Location	Loc X Coll[b]
Emergence Date[c]	56	57	NS	NS
GDD Emergence[d]	139	245	*	NS
GDD 50% Leaf Drop[d]	2902	3864	**	NS
Senescence Date[c]	190	205	**	**
Shoot Number	25	15	**	NS
Shoot Height (cm)	100	91	*	NS
Plot Area (cm^2)	19318	8915	**	NS
Total Shoot Dry Weight (g)	185	462	**	**
Petiole Length (mm)	2.1	5.0	**	*
Leaf Length (mm)	61	57	NS	NS
Leaf Width (mm)	23	25	NS	NS
Leaf Length/Width	2.73	2.37	**	NS

[a]* $P \leq 0.05$; ** $P \leq 0.01$; NS = no significant difference at $P \leq 0.05$.
[b]Loc X Coll = location by collection interaction
[c]Values represent days beginning March 1, 1991.
[d]Base 50 F growing degree days beginning March 1, 1991.

Table 3. Growth characteristics of hemp dogbane populations.

Collection	Emergence Date[a]	Heat accumulation		Senescence Date[a]	
		Emergence	50% Leaf Drop	MI	IL
	---Days---	Growing degree days[b]		---------Days---------	
M-101	50	151	3283	183	208
M-102	65	242	3380	187	197
M-105	52	143	3403	188	208
M-108	49	128	3400	191	197
M-109	61	193	3386	189	205
M-112	61	211	3408	200	214
M-118	59	194	3381	189	191
M-124	55	158	3381	190	195
I-102	57	186	3319	187	200
I-103	55	174	3396	195	207
I-107	63	263	3445	188	208
I-109	62	364	3385	183	212
I-115	47	116	3402	195	199
I-116	57	183	3393	193	211
I-122	54	173	3508	188	209
I-124	57	192	3403	189	226
LSD (0.05)	11	NS	63	5	13

[a]Emergence and senescence dates represent days beginning March 1, 1991.
[b]Base 50 F growing degree days beginning March 1, 1991.

Table 4. Cumulative shoot numbers expressed as percent of maximum number of shoots produced.

Collection	June	July	August	September	Maximum
	Cumulative shoot number				
	------------------------% of maximum-------------------------				Shoot no.
M-101	49	60	100	100	31
M-102	62	78	100	100	13
M-105	50	67	99	100	34
M-108	73	71	99	100	26
M-109	83	89	99	100	15
M-112	66	70	98	100	18
M-118	74	87	98	100	10
M-124	46	60	96	100	54
I-102	67	77	96	100	6
I-103	63	69	95	100	13
I-107	84	93	94	100	7
I-109	58	77	94	100	17
I-115	49	59	91	100	44
I-116	78	78	89	100	12
I-122	77	86	85	100	12
I-124	62	71	83	100	13
LSD (0.05)	24	22	NS	NS	

Table 5. Shoot measurements and plot area for hemp dogbane collections.[a]

Collection	Shoot measurements		Total shoot dry weight		Plot area
	Number	Height	MI	IL	
		---cm---	-------------g------------		---100 cm^2---
M-101	31	96	127	374	210
M-102	13	69	36	172	116
M-105	34	85	94	283	123
M-108	25	111	304	808	276
M-109	15	83	131	911	71
M-112	14	118	338	856	21
M-118	10	91	143	571	45
M-124	54	95	239	687	382
I-102	5	88	96	152	20
I-103	13	100	179	167	81
I-107	7	71	22	87	24
I-109	17	101	268	266	187
I-115	44	126	402	581	312
I-116	12	91	182	477	40
I-122	12	96	209	631	107
I-124	13	104	187	369	59
LSD (0.05)	18	15	173	350	183

[a]Shoot number and height were measured in August 1991. Plot area was measured in September 1991 and total shoot dry weight was taken in November 1991.

Table 6. Leaf characteristics and petiole length of hemp dogbane collections.[a]

	Petiole length		Leaf characteristics		
Collection	MI	IL	Length	Width	Length/width ratio
	----------mm----------				
M-101	2.5	6.3	53	26	2.1
M-102	0.8	3.0	50	19	2.7
M-105	1.3	3.3	52	24	2.2
M-108	2.3	5.0	54	19	2.9
M-109	1.3	5.3	63	22	2.9
M-112	3.0	5.3	61	23	2.6
M-118	2.5	5.5	71	24	2.9
M-124	2.3	4.5	65	25	2.6
I-102	3.3	6.5	67	24	2.8
I-103	1.8	5.3	60	29	2.1
I-107	2.5	5.0	51	25	2.1
I-109	0.3	1.8	54	27	2.0
I-115	1.8	5.0	59	18	3.3
I-116	1.5	4.5	56	21	2.7
I-122	2.0	4.3	56	25	2.3
I-124	4.5	9.3	72	28	2.6
LSD (0.05)	1.3	1.2	8	4	0.3

[a]Measurements were taken August 1991.

CHAPTER 3

ISOZYME AND RAPD VARIATION AMONG AND WITHIN HEMP DOGBANE (*Apocynum cannabinum*) POPULATIONS

ABSTRACT

Clonal individuals from 16 hemp dogbane populations with phenotypic variation were analyzed using isozyme and randomly amplified polymorphic DNA (RAPD) analysis. Plants originated from populations in Michigan and Illinois. Three known *Apocynum* species, spreading dogbane, hemp dogbane, and prairie dogbane, were also evaluated for comparison. Genetic distance among populations was more pronounced with isozyme analysis compared to RAPD analysis. Both isozyme and RAPD analysis data separated spreading dogbane from all other plants analyzed. Genetic variation was present among the 16 hemp dogbane populations, but was less than expected based on the phenotypic variation present among the collections. The short genetic distance between the 16 hemp dogbane collections and the three *Apocynum* species suggests that variation among populations of hemp dogbane may be from outcrossing with other closely related *Apocynum* species. Isozyme and RAPD analysis was also conducted on plants from two populations in Michigan to determine the level of genetic variation among plants within the same population. Genetic analysis revealed that one population was entirely clonal while the other population was a mixture of clonal and segregating plants.

Nomenclature: hemp dogbane, *Apocynum cannabinum* L. #[1] APPCA; prairie dogbane,

---

[1]Letters followed by this symbol are a WSSA-approved computer from Composite List of Weeds, Revised 1989. Available from WSSA, 1508 West University Ave., Champaign, IL 61821.

Apocynum sibericum Jacq. # APCVE; spreading dogbane, *Apocynum andromaesifolium* L. # APCAN.

Additional index words. Genetic variation, polymorphism, isozyme analysis, RAPD analysis, APPCA.

INTRODUCTION

Hemp dogbane is a perennial weed that is native to the United States and widely distributed in areas of the eastern and midwestern states (Anonymous 1970). Hemp dogbane can grow in dense patches within a crop causing significant yield losses (Shultz and Burnside 1979). The ability of hemp dogbane to grow in diverse environments (Balbach 1965) suggests it is either genetically diverse or highly adaptable. Genetic variation within hemp dogbane could come from its ability to cross with other *Apocynum* species (Anderson 1936; Balbach 1965; Woodson 1930). Hemp dogbane is also apparently self sterile (Anderson 1936; Balbach 1965; Woodson 1930) and cross pollination is likely frequent (Anderson 1936). Hemp dogbane morphology can be strongly influenced by soil type and fertility level (Robison and Jeffery 1972). However, even when grown in a common environment, plants collected from geographically isolated populations exhibited variability in growth and morphology (Chapter 1). Variation in morphological traits can have limited use in understanding population genetics because they are often limited in number, can be influenced by environmental conditions, and are often inherited by multiple genes (Nissen et. al 1995).

Nissen et. al (1995) recently described two molecular analysis techniques and their application to weed science. Isozyme and Randomly Amplified Polymorphic DNA (RAPD) analysis are two techniques that are used to examine genetic variation among and within plant species. These techniques can be more powerful than morphological markers because they allow the detection of heterogeneity among plants that appear morphologically identical (Anonymous 1991). These techniques can also be used to quantify the level of genetic variation present between plants with different phenotypes. Isozyme analysis has been used to examine genetic variation among and within populations of yellow nutsedge (Horak and Holt 1986; Horak et. al 1987), broom snakeweed (*Gutierrezia sarothrae* (Pursh) Britt. & Rusby) (Hou and Sterling 1995), purple loosestrife (*Lythrum salicaria* L.) (Strefeler et. al 1996), common lambsquarters (*Chenopodium album* L.) (Mouemar and Gasquez 1983), velvetleaf (*Abutilon theophrasti* Medicus), jimsonweed (*Datura stramonium* L.), wild proso millet (*Panicum miliaceum* L.), johnsongrass, and giant foxtail (*Setaria faberi* Herrm.) (Warwick 1990). Isozyme analysis has also been employed for the study of pollen flow in common milkweed (*Asclepias syriaca* L.) (Shore 1993). Considerable information about the genetic relationships among *Conyza* species (Thébaud and Abbott 1995) and among the *Setaria* species (Wang et. al 1995a; 1995b) and within the *Daucus carota* (St. Pierre et. al 1990) and *Carex pachystachya* (Whitkus 1992) complexes have been generated utilizing isozyme analysis. Some weaknesses of isozyme analysis are that a limited number of loci can be sampled and allozyme variation may be too low for analysis of genetic diversity within and among plant populations (Nissen et. al 1995).

RAPD analysis involves the amplification of small sequences of target DNA using random primers. As a DNA-based marker, the strength of RAPD analysis is the ability to assess genetic variation throughout the genome and generate a nearly limitless numbers of characters for evaluation (Nissen et. al 1995). This makes RAPD analysis a powerful tool for assessing genetic variation within and among weed populations. RAPD analysis has been used to analyze different populations of leafy spurge (*Euphorbia esula* L.) and may be important for determining the susceptibility of leafy spurge populations to biological control agents (Nissen et. al 1995). RAPD analysis has also been used to look at relationships among populations of cultivated and weedy eggplant (*Solanum melongena* L.) species (Karihaloo et. al 1995) and to examine genetic variation among and within populations of wild mustard (Sinapsis arvensis L.) (Moodie et. al 1997). RAPD analysis provides a powerful tool for studying all aspects of weed taxonomy and population genetics.

The objectives of this study were to use isozyme and RAPD analysis to, 1) determine the level of genetic variation among phenotypically variable populations of hemp dogbane, and 2) examine the genetic variation present within hemp dogbane populations.

MATERIALS AND METHODS

Among Population Variation

Clonal plants from 16 hemp dogbane populations evaluated in a common garden experiment were used to establish plants in the greenhouse. Plants were collected from populations in Illinois and Michigan (Table 1). Large differences in growth and morphology were observed among the 16 collections when they were grown in a common garden (Chapter 1).

For both isozyme and RAPD analysis, the youngest fully-expanded leaves were collected and used as a tissue source. Representative plants of three *Apocynum* species obtained from the Beal Botanical garden at Michigan State University were subjected to isozyme and RAPD analysis alongside plants from the 16 populations. The representative species were spreading dogbane, hemp dogbane, and prairie dogbane.

Within Population Variation

To examine the level of genetic variation present within hemp dogbane populations, 25 plants each were randomly collected from two geographically isolated populations of hemp dogbane in Michigan. One population was from Shiawassee County and the other from Kalamazoo County, Michigan. Plants were grown in the greenhouse and leaf tissue was used to conduct isozyme and RAPD analyses.

Isozyme Analysis

For isozyme analysis, the first fully expanded leaves were collected and placed between moist paper towels. Fresh leaf tissue was crushed in 125 ul of extraction buffer (75 m*M* Tris-HCl buffer, pH 7.5, 50 g/L polyvinylpyrrolidone-40, and 0.2% v/v 2-mercaptoethanol (14 m*M*) using a chilled plexiglass pestle and 12-sample porcelain plates. The extract was absorbed onto two 3 by 9 mm filter paper[2] wicks and immediately placed in a -20 C refrigerator. Two gel buffer systems were used to resolve

---

[2]Whatman No.3 Filter paper. Whatman International Ltd., Maidston, England.

isozyme banding patterns: a pH 5.7 histidine-citrate gel, and a pH 8.3 lithium-borate gel (Wendel and Weeden 1989). The gels consisted of 10.5% potato starch. The pH 5.7 Gel was electrophoresed at 35 mA for 5 h and the pH 8.3 gel was electrophoresed at 50 mA for 4 h. Gels were run in a 4 C cooler with ice bags placed on the gels to prevent heat buildup. Following electrophoresis, gels were removed from the gel trays and sliced horizontally into four slices. Each slice was assayed with a different enzyme specific stain. Phosphoglucose isomerase (PGI), phosphogluconate dehydrogenase (6-PGDH), malate dehydrogenase (MDH), and aconotase (ACO) enzyme systems were assayed using the histidine-citrate pH 5.7 buffer system, while phosphoglucomutase (PGM), aminoaspartate transaminase (GOT), diapherase (DIA), peroxidase (PRX), and triose phosphate isomerase (TPI) were resolved with the lithium-borate pH 8.3 buffer system. Enzyme staining techniques are described by Vallejos (1983). Once the stains were added, gels were incubated until banding patterns had adequately developed. Enzyme activity was stopped by rinsing the gels twice with water and fixing in 50% ethanol. Isozyme gels were repeated. Gels were evaluated and banding patterns recorded.

Randomly Amplified Polymorphic DNA (RAPD) Analysis

For RAPD analysis, young leaf tissue was collected from plants in the greenhouse, frozen at -80 C, and freeze-dried. DNA was extracted from 200 mg of freeze-dried leaf material using a CTAB DNA extraction described by (Dellaporta et. al 1983). The extraction was modified by adding a DNA purification procedure described by Li et. al (1994). DNA quantities were determined by DNA fluorometry and by visual comparison with known quantities of λ DNA on an agarose gel stained with EtdBr (0.5 μg/ml). DNA concentrations were adjusted to less than 100 ng/μl prior to use for RAPD analysis. Amplification was performed in a 25 μl reaction containing approximately 50 ng of genomic plant DNA template, 10 m*M* Tris-HCl, pH 8.3, 10 m*M* KCl, dATP, dCTP, dGTP, and dTTP (200 μM each), 5 m*M* $MgCl_2$, 2 μM random primer, and stoffel fragment enzyme[3] (2 units/100 μl). The primers used were 10-base primers from random primer kits[4] A, E, I, and X. The reactions were incubated in a DNA thermal cycler[5] with one cycle of 94 C for 2 min; 40 cycles of 92 C for 1'15", 37 C for 1'15", and 72 C for 2 min; followed by one cycle of 72 C for 5 min. Following amplification, 25 μl of each reaction were loaded on a 2% agarose gel with 250 ng of Hind III digested λ DNA loaded in the first lane as a size standard. Amplified fragments were separated at 50 V for 5 hours in 1x Tris- acetate (TAE) buffer (4 m*M* Tris-acetate, 1 m*M* EDTA). Gels contained EtdBr allowing DNA fragments to be visualized on a ultraviolet transilluminator. RAPD analyses were repeated for reactions producing polymorphic amplification products. Repeatable bands were rated as present or absent for all collections analyzed.

For both isozyme and RAPD analysis, genetic distance between collections and species was determined using Nei's distance formula (Nei 1972). Dendograms were created using the unweighted pair group method with arithmetic averages (UPGMA) cluster

---

[3]Perkin Elmer PCR reagents, Perkin Elmer Applied Biosystems Division, Foster City, CA 94404.

[4]Operon Technologies, Inc. Alameda, CA 94501.

[5]Perkin Elmer DNA thermal Cycler 480, Perkin Elmer Applied Biosystems Division, Foster City, CA 94404.

analysis. All genetic distance calculations and dendograms were made using NTSYS-pc version 1.7 software (Rohlf 1992).

RESULTS AND DISCUSSION

Variation Among Populations

The nine enzyme systems assayed revealed 12 allozymes, and seven of the allozymes were polymorphic (Table 2). The allozymes *Mdh*-2, *Pgi*-1, and *Tpi*-2 were polymorphic only among the three representative *Apocynum* species, but not among the 16 collections of hemp dogbane. Polymorphism was present among the 16 hemp dogbane collections for the remaining allozymes. Electrophoretic banding patterns for MDH and 6-PGDH are presented diagrammatically in Figure 1. The three *Apocynum* species had distinct electrophoretic patterns for *Mdh*-2 and 6-*Pgdh*-1, while polymorphic patterns were apparent among the sixteen hemp dogbane collections for 6-*Pgdh*-1 and 6-*Pgdh*-2. Genetic distance among the 16 populations as calculated from the isozyme data ranged from 0 to 0.302, while distance among the three different species ranged from 0.118 to 0.423 (data not shown).

For RAPD analysis, amplification was affected by the random primer used and some primers failed to amplify DNA bands. Of the 15 primers tested, 12 primers amplified bands for evaluation. Amplified DNA fragments ranged in size from 200 to 1500 base pairs. Amplification was inconsistent for collections 3, 13, and 16, with half of the amplified bands absent for these collections. The 12 primers generated 47 bands, and nine bands were polymorphic (Table 2). Figure 2 shows RAPD gels with polymorphic bands present among hemp dogbane populations for DNA sequences amplified with OPI-14 and OPI-18 primers. Polymorphic fragments amplified with OPI-14 are indicated by arrows labeled a, b, and c in gel A. Collection 3 does not have fragment a, but is the only collection with fragment b. Collection 4 does not have fragment a or b, while collection 5 has fragment a and not b. All plants analyzed have fragment c, except for spreading dogbane. For RAPD analysis with OPI-18 (gel B) one polymorphic fragment (d) was amplified. Fragment d was amplified only in five of the collections.

Genetic distance values were less than observed with isozyme analysis. For the RAPD data, genetic distance values among the 16 populations of hemp dogbane ranged from 0 to 0.091, and distance among the three species ranged from 0.055 to 0.187 (data not shown). Genetic distance was generally smaller between each of the 16 hemp dogbane collections and the three species than among the three *Apocynum* species. Because similar trends were observed with genetic distances calculated from the isozyme and the RAPD data, the data were combined and used to calculate genetic distance values (Table 3). The genetic distance among the 16 collection were less than 0.1, while the genetic distance between the 16 collections and spreading dogbane was generally greater than 0.1. Genetic distance was small between the representative hemp dogbane and spreading dogbane species as well as between these specimens and the 16 hemp dogbane collections. A dendogram (Figure 3) for the 16 populations of hemp dogbane and the three *Apocynum* species was created from the genetic distance matrix using UPGMA cluster analysis. Several branches are present on the dendogram with spreading dogbane and collection 2 separating from the other plants analyzed. Collection 12 is isolated on a branch as is prairie dogbane, while the hemp dogbane specimen from the botanical garden and the other hemp dogbane populations fall under the same broad cluster. Based on the morphological variation observed in the common garden experiment (Chapter 1), more genetic distance among the 16 populations of hemp dogbane was expected. However, genetic variation is present among the hemp dogbane collections examined and the clustering of the hemp dogbane collections with the three *Apocynum* species suggests that some hemp dogbane populations may be closely related with other *Apocynum* species. The lack of genetic distance between the hemp dogbane collections and prairie dogbane is not surprising since taxonomic separation of these species is viewed with skepticism (Voss 1996). Variability among hemp dogbane populations may be partially due to outcrossing with other closely related species. The genetic distance grouping of the 16 collections of hemp dogbane do not correlate with the geographic location from which the plants originated or with differences observed for morphological characteristics. Genetic analysis of hemp dogbane using isozyme and RAPD techniques was able to separate spreading dogbane from the other *Apocynum* species in the study. However, the spreading dogbane in this study was an isolated specimen and further analysis would be required to determine if spreading dogbane can consistently be distinguished from other *Apocynum* species using molecular markers.

Within Population Variation

In determining within population variability, seven enzyme systems were examined including DIA, GOT, MDH, 6-PGDH, PGI, PGM, and TPI. RAPD analysis was conducted with 9 random 10-base primers (OPA-02, OPA-04, OPA-05, OPA-15, OPE-07, OPE-18, OPE-20, and OPX-02). No variation was observed with either isozyme or RAPD analysis for the plants collected from the hemp dogbane population in Shiawassee County. In addition, all plants from the Shiawassee population were heterozygous for the 6-*Pgdh*-1 locus, suggesting they are all clones. For plants collected from Kalamazoo County population, isozyme analysis revealed variation at two loci (*Mdh*-2 and 6-*Pgdh*-1). For RAPD analysis of plants from the Kalamazoo County population, a single plant had two unique amplified bands with OPA-04 and OPE-18. For the two polymorphic isozyme loci, four possible isozyme phenotypes were identified (Table 4). The *Mdh*-2 'fast' allozyme did not segregate in a Mendelian fashion, but a relationship was observed between this locus and the polymorphic 6-*Pgdh*-1 locus. Plants with the 'slow' allozyme for *Mdh*-2 always were heterozygous for 6-*Pgdh*-1. Plants with a 'fast' allozyme for *Mdh*-2, had either the homozygous or heterozygote patterns of 6-*Pgdh*-1 typical of an interbreeding population. This suggests that the seven plants with the phenotype 1 are likely clonal and represent a proportion of the Kalamazoo County population that is clonal. The other plants have segregated for the 6-*Pgdh*-1 locus and likely represent a portion of the population that arose from outcrossing with adjacent populations or arose from more than one clone at the time the population became established. Further investigation could reveal the factors that determine if a hemp dogbane population is entirely clonal or made up of genetically variable individuals.

LITERATURE CITED

LITERATURE CITED

Anderson, E. 1936. An experimental study of hybridization on the genus *Apocynum*. Ann. Missouri Bot. Gard. 23:159-169.

Anonymous. 1991. An overview of new techniques and advances in weed physiology and molecular biology. Weed Sci. 39:480-481.

Anonymous. 1970. Selected Weeds of the United States. Agricultural handbook No. 336. Agricultural Research Service. United States Department of Agriculture. Pages 284-285.

Balbach, H. E. 1965. Variation and speciation in populations of Apocynaceae in North America. Ph.D. Thesis. Univ. Illinois at Urbana Champaign, Urbana, Ill.

Dellaporta, S. J., J. Wood, and J. B. Hicks. 1983. A plant DNA minipreparation: Version II. Plant Mol. Biol. Reporter. 1:19-20.

Horak, M. J., J. S. Holt, and N. C. Ellstrand. 1987. Genetic variation in yellow nutsedge (*Cyperus esculentus*). Weed Sci 35:506-512.

Horak, M. J., and J. S. Holt. 1986. Isozyme variability and breeding systems in populations of yellow nutsedge (*Cyperus esculentus*). Weed Sci 34:538-543.

Hou, Y., and T. M. Sterling. 1995. Isozyme variation in broom snakeweed (*Gutierrezia sarothrae*). Weed Sci. 43:156-162.

Karihaloo, J. L., S. Brauner, and L. D. Gottlieb. 1995. Random amplified polymorphic DNA variation in the eggplant, *Solanum melongena* L. (Solanaceae). Theor Appl. Genet. 90:767-770.

Li, Q., Q. Cai, and C. L. Guy. 1994. A DNA extraction method for RAPD analysis from plants rich in soluble polysaccharides. Plant Mol. Biol. Reporter. 12:215-220.

Moodie, M., R. P. Finch, and G. Marshall. 1997. Analysis of genetic variation in wild mustard (*Sinapsis arvensis*) using molecular markers. Weed Sci. 45:102-107.

Mouemar, A. A. and J. Gasquez. 1983. Environmental conditions and isozyme polymorphism in *Chenopodium album* L. Weed Res. 23:141-149.

Nei, M. 1972. Genetic distance between populations. Am. Nat. 106:283-292.

Nissen, S. J., R. A. Masters, D. J. Lee, and M. L. Rowe. 1995. DNA-based marker systems to determine genetic diversity of weedy species and their application to biocontrol. Weed Sci. 43:504-513.

Robinson, L. R. and L. S. Jeffery. 1972. Hemp dogbane growth and control. Weed Sci. 20:156-159.

Rohlf, F. J. 1992. NTSYS-pc. Numerical taxonomy and multivariate analysis system, version 1.7. Exeter Software, NewYork.

Schultz, M. E. and O. C. Burnside. 1979. Distribution, competition, and phenology of hemp dogbane (*Apocynum cannabinum*) in Nebraska. Weed Sci. 27:565-570.

Shore, J. S. 1993. Pollination genetics of common milkweed, *Asclepias syriaca* L. Hered. 70:101-108.

St. Pierre, M. D., R. J. Bayer, and I. M. Weis. 1990. An isozyme-based assessment of the genetic variability within the Daucus carota complex (Apiaceae: Caucalidae). Can. J. Bot. 68:24449-2457.

Strefeler, M. S., E. Darmo, R. L. Becker, and E. J. Katovich. 1996. Isozyme characterization of genetic diversity in Minnesota populations of purple loosestrife, *Lythrum salicaria* (Lythraceae). Amer. J. Bot. 83:265-273.

Thébaud, C. and R. J. Abbott. 1995. Characterization of invasive *Conyza* species (Asteraceae) in Europe: Quantitative trait and isozyme analysis. Amer. J. Bot. 82:360-368.

Vallejos, E. 1983. Enzyme activity staining. In S. D. Tanksley and T. J. Norton (eds.) Isozymes in plant genetics and breeding, Part A, 469-516, Elsevier, Amstradam.

Voss, E. G. 1996. Michigan flora: Part III. Dicots (Pyrolaceae-Compositae). Cranbrook Institute of Science Bulletin 61 and University of Michigan Herbarium. pp. 84-87.

Wang, R. L., J. F. Wendel, and J. H. Dekker. 1995. Weedy adaptation in *Setaria* spp. I. Isozyme analysis of genetic diversity and population genetic structure in *Setaria viridis*. Amer. J. Bot. 82:308-317.

Wang, R. L., J. F. Wendel, and J. H. Dekker. 1995. Weedy adaptation in *Setaria* spp. II. Genetic diversity and population genetic structure in *S. glauca*, *S. geniulata*, and *S. faberii* (Poaceae). Amer. J. Bot. 82:1031-1039.

Warwick, S. I. 1990. Allozyme and life history variaiton in five northwardly colonizing North American weed species. Plant Syst. Evol. 169: 41-54.

Wendel, J. F. and N. F. Weeden. 1989. Visualization and interpetation of plant isozymes. Pages 4-45 *in* D. E. Soltis and P. S. Soltis eds., Isozymes in plant biology. Dioscorides Press, Portland, OR. 4-45 pp.

Whitkus, R. 1992. Allozyme variation within the *Carex pachystachya* complex (Cyperaceae). Syst. Bot. 17:16-24.

Woodson, R. E. Jr. 1930. Studies in the Apocynaceae I. Ann. Missouri Bot. Gard. 17:1-212.

Table 1. County and state of origin of hemp dogbane collections.

Collection		Collection area	
Number	Code	County	State
1	M-101	Shiawassee	Michigan
2	M-102	Huron	Michigan
3	M-105	Arenac	Michigan
4	M-108	Cass	Michigan
5	M-109	Monroe	Michigan
6	M-112	Kalamazoo	Michigan
7	M-118	Allegan	Michigan
8	M-124	Tuscola	Michigan
9	I-102	Jackson	Illinois
10	I-103	Adams	Illinois
11	I-107	Woodford	Illinois
12	I-109	Iroquois	Illinois
13	I-115	LaSalle	Illinois
14	I-116	LaSalle	Illinois
15	I-122	Livingston	Illinois
16	I-124	Randolph	Illinois

Table 2. Isozyme enzyme systems and RAPD primers used for analysis of hemp dogbane populations.

Isozyme analysis			RAPD analysis		
Enzyme	Number of loci	Number that are polymorphic	Primer	Number of bands	Number that are polymorphic
ACO	1	0	OPA-01	3	0
DIA	1	1	OPA-02	5	1
GOT	1	0	OPA-03	4	1
MDH	2	1	OPA-09	4	2
6-PGDH	2	2	OPA-10	3	0
PGI	1	1	OPA-11	2	1
PGM	1	1	OPA-12	5	0
PRX	1	0	OPA-13	6	0
TPI	2	1	OPA-14	5	3
Total	12	7	OPA-15	3	0
			OPA-16	5	0
			OPA-18	2	1
			Total	47	9

Table 3. Matrix of Nei's coefficients of genetic distance for RAPD and isozyme data for 16 collections of hemp dogbane and three *Apocynum* species.

	Hemp dogbane collections[a]																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	A. a.	A. c.
2	0.140																	
3	0.030	0.137																
4	0.052	0.066	0.039															
5	0.064	0.112	0.032	0.022														
6	0.064	0.066	0.051	0.011	0.011													
7	0.038	0.112	0.038	0.034	0.022	0.022												
8	0.058	0.078	0.038	0.017	0.006	0.005	0.016											
9	0.045	0.102	0.032	0.028	0.011	0.016	0.005	0.005										
10	0.040	0.095	0.033	0.012	0.029	0.023	0.023	0.023	0.023									
11	0.032	0.141	0.060	0.052	0.040	0.040	0.017	0.034	0.023	0.042								
12	0.091	0.066	0.084	0.045	0.045	0.033	0.044	0.027	0.033	0.052	0.063							
13	0.026	0.102	0.008	0.025	0.017	0.025	0.008	0.008	0.000	0.017	0.034	0.051						
14	0.064	0.066	0.051	0.011	0.011	0.000	0.022	0.005	0.016	0.023	0.040	0.033	0.025					
15	0.040	0.118	0.041	0.035	0.023	0.023	0.000	0.017	0.006	0.024	0.018	0.047	0.009	0.023				
16	0.070	0.114	0.028	0.049	0.029	0.047	0.047	0.019	0.019	0.031	0.060	0.066	0.019	0.047	0.053			
A. a.	0.186	0.053	0.156	0.114	0.156	0.130	0.130	0.130	0.130	0.136	0.152	0.119	0.130	0.130	0.098	0.152		
A. c.	0.049	0.086	0.042	0.018	0.012	0.006	0.006	0.006	0.006	0.031	0.024	0.036	0.009	0.006	0.006	0.022	0.130	
A. s.	0.05	0.196	0.045	0.033	0.039	0.045	0.045	0.045	0.045	0.020	0.040	0.092	0.054	0.045	0.049	0.050	0.195	0.055

[a]Abbreviations: A.a., *Apocynum androsaemifolium* L.; A.c., *Apocynum cannabinum* L.; A.s., *Apocynum sibericum* Jacq.

Table 4. Isozyme phenotypes for plants collected within the Kalamazoo County population.

	Allelic pattern		
Phenotype	*Mdh*-2[a]	6-*Pgdh*-1[b]	Individuals with phenotype
			-----Number-----
1	S	FS	7
2	F	FS	2
3	F	F	3
4	F	S	7

[a]F, fast allozyme; S, slow allozyme.
[b]F, 'fast' homozygote; S, 'slow' homozygote; FS, heterozygote.

Figure 1. Schematic diagram of isozyme patterns for MDH and PGDH for 16 populations of hemp dogbane (1 to 16) and three *Apocynum* species. Representative species are ; Aa, *Apocynum andraemisifolium* L.; Ac, *Apocynum cannabinum* L.; As, *Apocynum sibericum* Jacq. The origin (-) is at the bottom and the front (+) at the top of both electrophoretic diagrams.

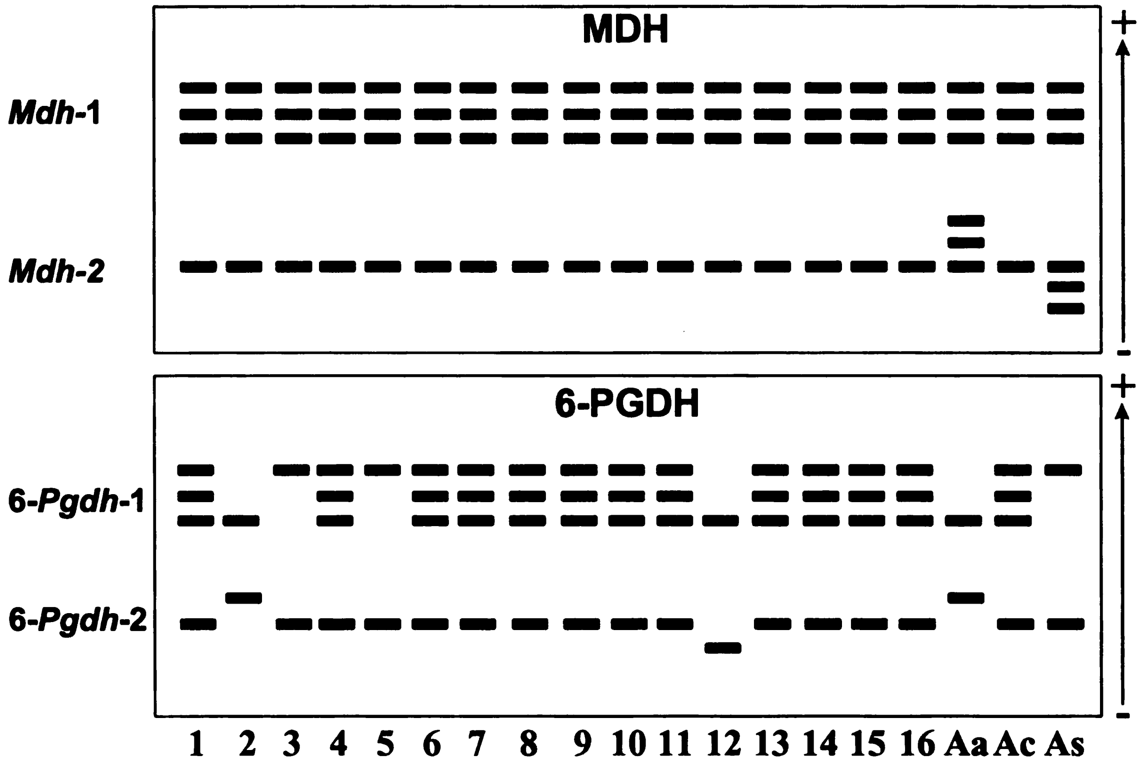
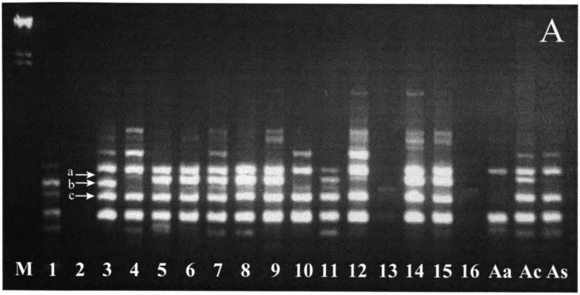

MDH
Mdh-1
Mdh-2
6-PGDH
6-Pgdh-1
6-Pgdh-2
+
-
+
-
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 Aa Ac As

Figure 2. Genomic DNA fragments from 16 populations of hemp dogbane (1 to 16) and three *Apocynum* species amplified with A) OPI-14 and B) OPI-18 primers. Representative species are ; Aa, *Apocynum andraemisifolium* L.; Ac, *Apocynum cannabinum* L.; As, *Apocynum sibericum* Jacq. Arrows labeled with a, b, and c in OPI-14 (gel A) and d in OPI-18 (gel B) represent amplified DNA fragments that are polymorphic among plants.


A
a
b
c
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 Aa Ac As

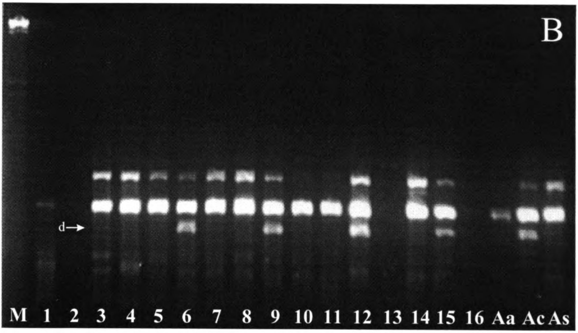
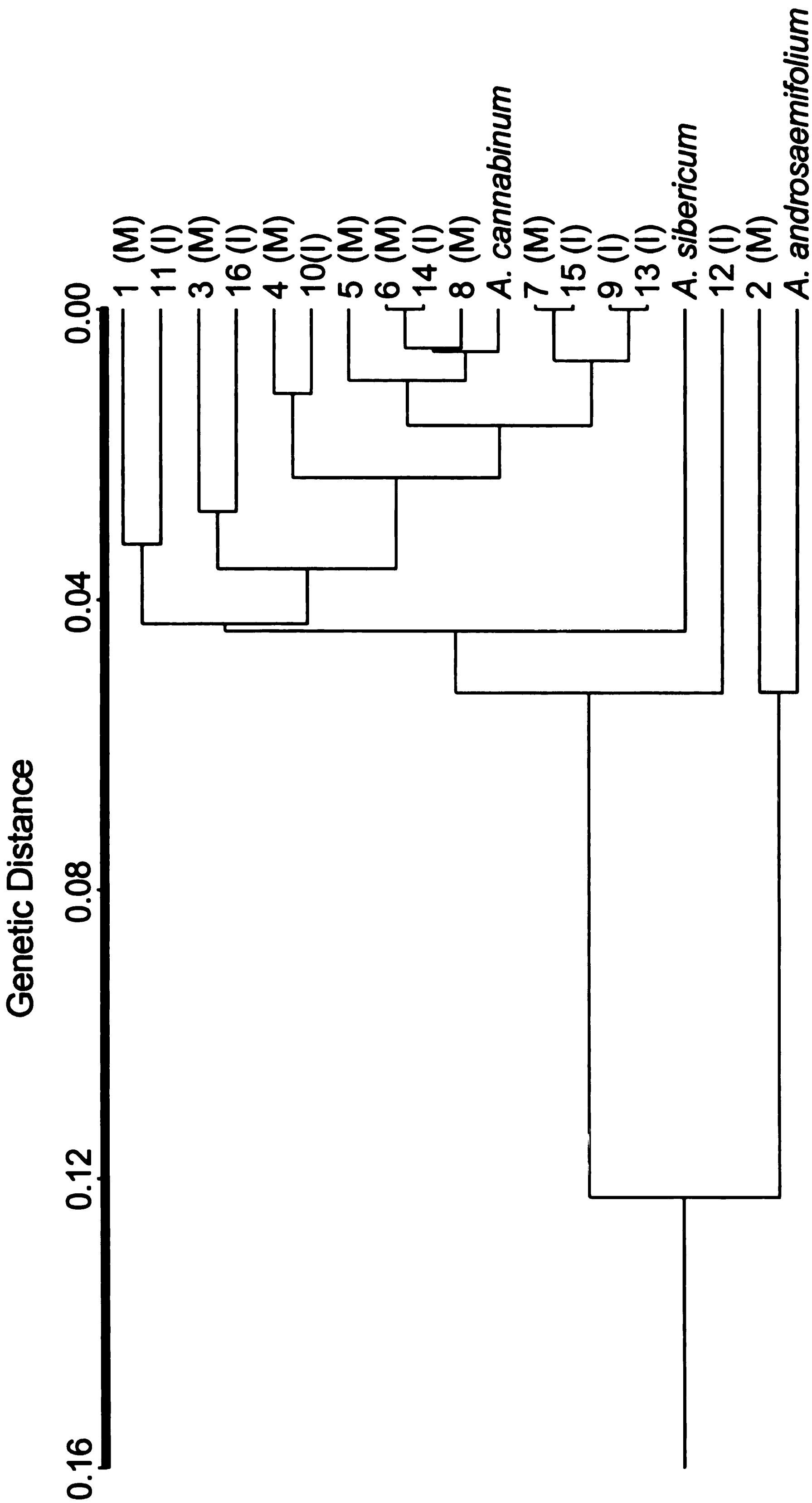

B
d
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 Aa Ac As

Figure 3. Dendogram based on Nei's genetic distance for 16 collections (1 to 16) of hemp dogbane and three *Apocynum* species generated by UPGMA cluster analysis. Collection numbers are followed by an (M) if they were collected in Michigan, and (I) if collected in Illinois.


Genetic Distance
0.00
0.04
0.08
0.12
0.16
1 (M)
11 (I)
3 (M)
16 (I)
4 (M)
10(I)
5 (M)
6 (M)
14 (I)
8 (M)
A. cannabinum
7 (M)
15 (I)
9 (I)
13 (I)
A. sibericum
12 (I)
2 (M)
A. androsaemifolium

CHAPTER 4

POSTEMERGENCE CONTROL OF HEMP DOGBANE (*Apocynum cannabinum*) IN CORN (*Zea mays* L.)

ABSTRACT

Field studies were conducted from 1994 to 1996 in Michigan to evaluate postemergence herbicides for hemp dogbane control in corn. Studies were initiated at no-tillage and chisel tillage sites each of the 3 years. Nicosulfuron and primisulfuron were evaluated alone and in combination with 2,4-D amine or dicamba. In 1995 and 1996, prosulfuron + primisulfuron was also applied alone and in combination with 2,4-D or dicamba. Nonionic surfactant was added to all treatments containing nicosulfuron, primisulfuron or prosulfuron + primisulfuron. Control varied between years and sites. Nicosulfuron, primisulfuron, and prosulfuron + primisulfuron applied alone provided an average of 30% control. Dicamba and 2,4-D applied alone were the most variable treatments with an average of 42% and 66% control, respectively. Tank mixtures of nicosulfuron, primisulfuron, or prosulfuron + primisulfuron with dicamba were more effective and more consistent than dicamba alone. Combinations of nicosulfuron, primisulfuron, or prosulfuron + primisulfuron with 2,4-D gave the most effective and consistent results across sites with an average of 93% control. In general, treatments controlled only shoots emerged at the time of application. New shoots emerged following herbicide application at the tilled sites in 1994 and 1995 but not at the no-tillage sites. However, in 1996 shoot emergence following treatment at the no-tillage site was greater than at the tilled site. **Nomenclature:** Dicamba, 3,6-dichloro-2-methoxybenzoic acid; nicosulfuron, 2-[[[[(4,6-dimethoxy-pyrimidinyl)amino]

carbonyl]amino]sulfonyl]-*N,N*-dimethyl-3-pyridine carboxamide; primisulfuron, 2-[[[[[4,6-bis(difluoromethoxy)-2-pyrimidinyl]amino]carbonyl]amino]sulfonyl]benzoic acid; prosulfuron, 1-(4-methoxy-6-methyl-triazin-2-yl)-3-[2-(3,3,3-trifuoropropyl)-phenylsulfonyl]-urea; 2,4-D, (2,4-, 2,4-dichlorophenoxy) acetic acid; hemp dogbane, *Apocynum cannabinum* L. #[6] APPCA; Corn, *Zea mays* L., # ZEAMX.

INTRODUCTION

Hemp dogbane is native to the United States and can be found throughout most of North America (Balbach 1965; Becker 1981; Woodson 1930). It can grow in diverse environments (Balbach 1965) and its growth habit is strongly affected by environmental conditions (Woodson 1930). The highest density infestations of hemp dogbane are generally found in low-lying, wet areas, but infestations are also present on drier upland soils (Becker 1981). A survey of 60 irrigated corn fields in eastern Nebraska found that of the fields surveyed, 40% had infestations of hemp dogbane (Evetts and Burnside 1973). Farmers participating in a survey in Ohio ranked hemp dogbane as the third most serious perennial broadleaf weed (Loux and Berry 1991). Shultz and Burnside (1979b) observed that hemp dogbane was present in all cropping systems, but was most prevalent in oats (*Avena sativa* L.) and soybeans, while least prevalent in alfalfa (*Medicago sativa* L.), pasture and wheat (*Triticum sativa* L.). They reported that hemp dogbane reduced corn (*Zea mays* L.) yields by 8 to 10% and soybean (*Glycine max* L.) and sorghum

---

[6]Letters following this symbol are WSSA-approved computer code from Composite List of Weeds, Weed Sci. 32, Suppl. 2. Available from WSSA, 1508 W. University Ave., Champaign, IL 61821-3133.

(*Sorghum bicolor* L.) yields by as much as 41 and 45% respectively. The author has observed hemp dogbane patches growing to the exclusion of crop plants suggesting that in well established hemp dogbane infestations yield losses are probably higher than reported in the literature.

The spread of hemp dogbane infestations within a field appears to be mainly by growth of underground rootstocks since hemp dogbane seed viability is relatively short (Burnside et. al 1996; Burnside et al 1981; Evetts and Burnside 1972). Hemp dogbane is increasing as a problem in Michigan crops possibly due to increases in the number of acres in no-tillage. Hemp dogbane infestations increase as tillage decreases (Loux and Berry 1991; Triplett 1985; Triplett and Lytle 1972). Tillage may reduce the density of established hemp dogbane populations, but it may also increase the area infested by breaking the roots into small pieces and moving them throughout the tilled area (Robinson and Jeffery 1972; Shultz and Burnside 1979a; Woodson 1930). As early as 1930, Woodson (1930) recognized hemp dogbane as becoming a pest of agriculture through its spread by the plowshare in cultivated fields. In corn, hemp dogbane densities increased as preplant tillage was decreased (Buhler et. al 1994). Hemp dogbane increases in reduced tillage systems because the underground roots are not disturbed and postemergence herbicide applications often do not correspond with the susceptible stage of hemp dogbane (Triplett 1985). In a long term tillage study, Triplett and Lytle (1972) reported that hemp dogbane became a serious problem, invading numerous plots while it did not invade tilled plots. Once established, herbicide treatments had little affect on hemp dogbane patches (Triplett and Lytle 1972). Buhler et. al (1994) observed that in a continuous corn rotation, hemp dogbane density was greater in no-tillage plots than in chisel or moldboard plowed plots. Crop rotation within the three tillage systems did not affect shoot densities of hemp dogbane. Shultz and Burnside (1979b) observed that shoots appear to emerge in the spring in response to soil temperature with shoots of hemp dogbane growing in a tilled nursery and in a sod waterway emerging when the soil temperature at the crown was 17 to 19 C.

Glyphosate (*N*-phosphonomethyl)glycine) can effectively control hemp dogbane in non-crop situations (Barnes and Brenchley 1972; Buhler et al 1994; Shultz and Burnside 1979b) and is most effective when applied in the fall (Barnes and Brenchley 1972; Shultz and Burnside 1979b) or at the late vegetative growth stage (Barnes and Brenchley 1972). More recently, glyphosate has become available for postemergence control of hemp dogbane in glyphosate resistant soybeans. Doll (1997) demonstrated that postemergence applications of glyphosate at rates higher than 0.84 kg/ha provided greater than 90% hemp dogbane control in glyphosate resistant soybeans. In the past most postemergence herbicides with significant activity on hemp dogbane were limited to use in corn. Shultz and Burnside (1979a) demonstrated that hemp dogbane emergence from crown and lateral roots could be suppressed or controlled (60 to 98%) up to 2 months following treatment using preemergence applications of thiocarbamate herbicides. However, control decreased (0 to 40%) by harvest.

Postemergence applications of 2,4-D in corn can provide good control of hemp dogbane (Glenn and Anderson 1993; Orfanedes and Wax 1991; Robinson and Jeffery 1972; Shultz and Burnside 1979a). Control is greatest when 2,4-D is applied at the late vegetative stage or in the fall as compared to spring applications (Orfanedes and Wax 1991; Robinson and Jeffery 1972). The ester formulations of 2,4-D generally provide greater control of hemp dogbane than the amine formulation (Shultz and Burnside 1979a). However, control with 2,4-D has been inconsistent from year to year (Glenn and Anderson 1993; Shultz and Burnside 1979a). Shultz and Burnside (1979a) also noted that farmers reported that hemp dogbane control with glyphosate and 2,4-D was erratic ranging from poor to excellent. Dicamba is generally applied postemergence in corn at half the rate of 2,4-D amine, and generally provides less control than 2,4-D (Glenn and Anderson 1993). Dicamba is also more effective when applied in the fall than in the spring (Robinson and Jeffery 1972). Glenn and Anderson (1993) reported that nicosulfuron alone provided greater than 67% hemp dogbane control, while tank mixtures containing nicosulfuron and 2,4-D or nicosulfuron and dicamba, provided 72 to 100% hemp dogbane control. Hemp dogbane control with 2,4-D (560 g ai/ha) and dicamba (280 g ai/ha) applied alone was variable between years and ranged from 63 to 97% for 2,4-D and 55 to 70% for dicamba.

Orfanedes and Wax (1991) observed resprouting of hemp dogbane plants near the base of controlled shoots 4 to 8 weeks after applications of fluoxypyr ([(4-amino-3,5-dichloro-6-flouro-2-pyridinyl)oxy]acetic acid). Regrowth also appeared to be greater for treatments applied at the vegetative stage as compared to the early reproductive growth stage. Increased regrowth from treatments applied at the vegetative growth stage may be due to incomplete emergence of hemp dogbane shoots at the time of application, or because translocation is acropetal during the vegetative growth preventing translocation of herbicide to the roots (Orfanedes and Wax 1991). Inconsistent control of hemp dogbane in the literature may be a result of emergence of additional shoots following herbicide application or differences in the activity of the herbicide between years or locations. The basis of inconsistent control of hemp dogbane has not been adequately described in the literature. The objectives of this study were to 1) identify effective postemergence herbicide strategies for control of hemp dogbane in corn and, 2) to examine the effect of tillage on hemp dogbane control.

MATERIALS AND METHODS

Hemp Dogbane Control with Tank Mixtures

Field studies were conducted from 1994 to 1996 in Michigan to evaluate postemergence herbicides for hemp dogbane control in corn. Studies were initiated at no-tillage and tilled sites each of the three years. Site descriptions of the tilled and no-tillage sites are shown in Table 1. Applications were made to tilled sites around the second week of June each year and approximately 2 weeks later at the no-tillage sites within each year. The three tilled sites had been chisel plowed in the spring and preemergence herbicides were applied after planting. At the no-tillage sites, a burndown of glyphosate or 2,4-D was applied prior to planting and preemergence herbicides were applied after corn planting. Accent (18 and 35 g/ha) and primisulfuron (20 and 40 g/ha) were evaluated alone and in combination with 2,4-D amine (560 g/ha) or dicamba (280 g/ha). In 1995 and 1996, prosulfuron + primisulfuron at two rates (20 and 40 g/ha) was also applied alone and in combination with 2,4-D or dicamba. Nonionic surfactant[7] (0.25% v/v) was added to all treatments containing nicosulfuron, primisulfuron or prosulfuron + primisulfuron. Dicamba and 2,4-D were also applied alone. Treatments were applied at 187 L/ha in 1994 and 205 L/ha in 1995 and 1996 and were delivered at 207 kPa pressure. Control of treated shoots was evaluated visually at 2, 4, 6, and 8 weeks after treatment (WAT). Because preemergence herbicides failed toward the end of the growing season at the 1994 tilled site, the last rating taken at this site was 6 WAT. In 1995 and 1996, in addition to evaluating the treated shoots in each plot, a second overall rating was used to account for the emergence of new shoots following application of the treatments.

Comparison of 2,4-D Formulations

Experiments were conducted from 1994 to 1996 to compare two amine and two ester formulations of 2,4-D for postemergence control of hemp dogbane in corn. All treatments were applied at 560 g/ha. The 1994 study was conducted on a no-tillage site in Barry County and treatments were applied June 21. At the time of application the corn was 20 cm tall and the hemp dogbane was 76 cm tall. The 1995 site was a tilled site in Cass County. At this site, treatments were applied to 23 cm corn and 53 cm hemp dogbane on June 12, 1995. The 1996 location was a no-tillage site in Kalamazoo county. Applications were made June 27, 1996 when the hemp dogbane was 56 cm tall and corn averaged 30 cm in height. Hemp dogbane densities at the three sites ranged from 28 to 36 plants/m^2.

---

[7]X-77 Spreader, alkylarylpolyoxythylene glycols, free fatty acids, isopropanol. Valent USA Corp., 1333 North California Blvd., Walnut Creek, CA 94596.

Statistical Procedures. In all studies treatments were arranged in a randomized complete block design with three or more replications. Data were analyzed using ANOVA and means were separated using Fishers protected LSD at the 5% confidence level. Arc sine transformations were calculated for control data, but transformed data did not affect the conclusions of this paper, so untransformed data are presented. Significant treatment by year and treatment by location interactions were present, so means are presented individually for all six studies. For an overall estimate of the level and consistency of control provided by each treatment, data from all six sites where the tank mixture study was conducted were combined and presented in a boxplot diagram.

RESULTS AND DISCUSSION

Hemp Dogbane Control with Tank Mixtures

Response of hemp dogbane to postemergence herbicide treatments varied among sites and years. Because few differences were observed in the activity of the high and low rates of the ALS inhibitor herbicides, only the low rates evaluated will be presented. Tank mixtures of nicosulfuron or primisulfuron with 2,4-D provided greater than 92% hemp dogbane control 8 WAT (Table 2) at five of the six locations, and 88% or grater control 6 WAT at the 1994 tilled site. Tank mixtures of prosulfuron + primisulfuron with 2,4-D also provided greater than 95% control in 1995 and 1996. Tank mixtures of nicosulfuron, primisulfuron, or prosulfuron + primisulfuron with dicamba provided control similar to the tank mixtures with 2,4-D at three of the locations, but significantly less control than the 2,4-D combinations at the other three locations. At the no-tillage sites in 1995 and 1996 and at the tilled site in 1995 nearly all treatments containing either 2,4-D or dicamba provided greater than 87%. At the other sites control with the growth regulator herbicides applied alone or in combinations with each other was variable and ranged from 15 to 83%. The variability in control with 2,4-D alone and dicamba alone is similar to the inconsistent control reported by Glenn and Anderson (1993). In this study, we observed that hemp dogbane response to herbicide applications was variable among the different sites, independent of shoot emergence after treatment,. Applications of nicosulfuron, primisulfuron, and prosulfuron + primisulfuron alone provided 62 to 74% control of hemp dogbane at the 1995 tilled site, but less than 38% at all other sites. Glenn and Anderson (1993) reported 85 to 90% control with nicosulfuron (31 g/ha) applied with 1% crop oil concentrate (COC). The level of control observed from ALS inhibitors applied alone in our studies was much lower than those reported by Glenn and Anderson (1993) even when applied at similar rates.

Control ratings for all six locations 6 WAT are combined in a boxplot diagram (Figure 1) to illustrate the level and consistency of control provided by treatments across years and locations. For this boxplot, the black dot represents the mean control while the box represents 50% of all the data and the whiskers represent 80% of the data. Shorter boxes and shorter whiskers represent more consistent control across years and locations. Nicosulfuron, primisulfuron, and prosulfuron + primisulfuron applied alone provided an average of less than 33% control 6 WAT and control ratings varied by as much as 60% for the 80% of the charted. Dicamba and 2,4-D alone were the most variable treatments with dicamba providing an average of 42% control and 2,4-D an average of 66% control.

Tank mixtures of nicosulfuron, primisulfuron, or prosulfuron + primisulfuron with dicamba were more consistent than 2,4-D or dicamba alone providing with an average of 68% control. Combinations of nicosulfuron, primisulfuron, or prosulfuron + primisulfuron with 2,4-D gave an average of 93% control and were the most consistent across sites as indicated by the small boxes and short whiskers in the boxplot diagram.

At the tilled site in 1994, additional shoots emerged in plots following the application of herbicide treatments. The newly emerged shoots were able to grow unaffected by the herbicide treatments. Few shoots emerged following herbicide application the 1994 no-tillage site. In 1995, each plot was evaluated with two ratings. For the first rating only treated shoots were evaluated. This accounted for the effect of the herbicide on shoots directly exposed to the treatment. The second rating evaluated both those shoots present at the time of treatment and shoots that had emerged after the treatments were applied. The two evaluations taken in plots treated with the tank mixture of primisulfuron with 2,4-D at the tilled and no-tillage sites in 1995 and 1996 are graphically in Figure 2. While the control of treated shoots with this tank mixture was similar between the four sites regardless of tillage, the emergence of shoots was different. In 1995, the two ratings were significantly different at 4 and 8 WAT (Graph A) indicating new shoot emergence at the tilled site, but the ratings were not different at the no-tillage site (Graph B). Doll (1997) also observed that hemp dogbane reinfestation following treatment with postemergence herbicides was less in no-tillage fields than in chisel tilled fields. However, in 1996 shoots emerged following treatment regardless of the tillage system. Significant differences were present between the two ratings at the no-tillage site (Graph

D) when evaluated 2, 4, and 8 WAT. New shoots also emerged at the 1996 tilled site (Chart C), but a high level of variability at the site prevented the differences between the two ratings from being statistically significant. The effect of new emerging shoots on hemp dogbane control suggests that even postemergence herbicides with excellent activity on hemp dogbane control only shoots that are present at the time of application. Overall control is then highly dependant on the emergence patterns of the plants being treated. Emergence of hemp dogbane shoots following herbicide treatment was reported by Orfanedes and Wax (1991). The absence of new shoot emergence at the no-tillage sites in 2 of the 3 years suggest that a no-tillage system may provide more uniform emergence of hemp dogbane shoots, and therefore may allow for greater control of hemp dogbane with postemergence treatments.

Biological factors may account for the differences in emergence of shoots following treatment observed at the 1996 no-tillage site in comparison to the 1994 and 1995 no-tillage sites. In prior research at Michigan State University comparing the growth and development of different for populations of hemp dogbane, populations were observed with different emergence patterns even when grown under the same environmental conditions (Chapter 1). The presence of a population with a delayed emergence pattern could explain the differences observed between the no-tillage sites.

Environmental factors could also influence the hemp dogbane emergence. Environmental differences among the no-tillage sites include the number of years the sites were in no-tillage, growing degree day (GDD) accumulation prior to treatment, and rainfall 6 weeks prior to application (Table 3). The accumulation of GDD does not explain the different emergence pattern of hemp dogbane plants, since the 1996 location had an intermediate level of GDD in comparison to the other sites. The shorter amount of time in no-tillage and the increased rainfall prior to treatment at the 1996 no-tillage site may have affected hemp dogbane emergence, resulting in emergence of shoots following the application of treatments. Tillage the previous year may have affected hemp dogbane emergence by breaking the roots into pieces and distributing them to various depths in the soil profile, thus extending the period of shoot emergence. The additional rainfall may have delayed the warming of the soil and subsequently delayed the emergence of the hemp dogbane plants. Since tilled and no-tillage sites in this study were at different locations and under different environmental conditions, direct comparisons of the effects of tillage are limited. Research comparing tilled and no-tillage plots within the same experiment are needed to fully explain the effect of tillage on postemergence control of hemp dogbane.

Comparison of 2,4-D Formulations

Hemp dogbane control 28 DAT was greater with ester formulations than with the amine formulations of 2,4-D. By 56 DAT there were no difference among formulations at the 1995 site, but ester formulations still provided greater control than amine formulations at the 1994 and 1996 sites. The results of this study agree with earlier research showing that ester formulations of 2,4-D generally are more active on hemp dogbane than amine formulations (Shultz and Burnside 1979). In addition, the 2,4-D formulation containing a combination of the dimethyl amine and diethanol amine salts of 2,4-D did not provide control different from that provided by the formulation containing only the dimethyl amine salt of 2,4-D. Our research showed no differences in hemp dogbane control between the two amine formulations or between the two ester formulations (Table 4).

LITERATURE CITED

LITERATURE CITED

Balbach, H. E. 1965. Variation and speciation in populations of Apocynaceae in North America. Ph.D. Thesis. Univ. Illinois at Urbana Champaign, Urbana, Ill.

Barnes, D. and R. Brenchley. 1972. Response of hemp dogbane to the isopropylamine salt of glyphosate (MON-2139). Proceedings Central Weed Control Conf. 27:54-55.

Becker, R. 1981. Todays Weed: hemp dogbane. Weeds Today. Winter Issue. pp. 15-16.

Buhler, D.B., D.E. Stoltenberg, R. L. Becker, and J. L. Gunsolus. 1994. Perennial Weed Populations after 14 years of variable tillage and cropping practices. Weed Sci. 42:205-209.

Burnside, O. C., R. G. Wilson, S. Weisberg, and K. G. Hubbard. 1996. Seed longevity of 41 weed species buried 17 years in eastern and western Nebraska. Weed Sci. 44:74-86

Burnside, O. C., C. R. Fenster, L. L. Evetts, and R. F. Mumm. 1981. Germination of exhumed weed seed in Nebraska. Weed Sci. 29:577-586

Costa, J. and A. P. Appleby. 1976. Response of two yellow nutsedge varieties to three herbicides. Weed Sci. 24: 54-58.

Doll, J. D. 1997. Hemp dogbane (Apocynum cannabinum L.) management in corn and gyphosate-resistant soybeans. Abstr. Weed Sci. Soc. Am. 37:90.

Evetts, L. C. and O. C. Burnside. 1972. Germination and seedling development of common milkweed and other species. Weed Sci. 20:371-378.

Evetts, L. C. and O. C. Burnside. 1973. Watch out for hemp dogbane. Nebraska Farm, Ranch, and Home Quart. 19:19-20.

Glenn, S. and N. G. Anderson. 1993. Hemp dogbane (*Apocynum cannabinum*) and wild blackberry (*Rubus allegheniensis*) control in no-tillage corn (*Zea mays*). Weed Technol. 7:47-51.

Loux M. M. and M. A. Berry. 1991. Use of a grower survey for estimating weed problems. Weed Technol. 5:460-66.

Orfanedes, M.S. and L.M. Wax. 1991. Differential response of hemp dogbane (*Apocynum cannabinum*) to clopyralid, dowco 433, and 2,4-D. Weed Technol. 5:782-788.

Robinson, L. R. and L. S. Jeffery. 1972. Hemp dogbane growth and control. Weed Sci. 20:156-159.

Schultz, M. E. and O. C. Burnside. 1979a. Control of hemp dogbane with foliar and soil applied herbicides. Agron. J. 71:723-729.

Schultz, M. E. and O. C. Burnside. 1979b. Distribution, competition, and phenology of hemp dogbane (*Apocynum cannabinum*) in Nebraska. Weed Sci. 27:565-570.

Triplett, G. B. Jr. 1985. Principles of weed control for reduced-tillage corn production. Pages 26-40 *in* A. F. Weise, ed. Weed control in limited tillage systems. Weed Sci. Soc. Am. Mongr. 2, Champaign, IL.

Triplett, G. B. Jr. and G. D. Lytle. 1972. Control and ecology of weeds in continuous corn grown without tillage. Weed Sci. 20:453-457.

Woodson, R. E. Jr. 1930. Studies in the Apocynaceae I. Ann. Missouri Bot. Gard. 17:1-212.

Table 1. Site description for study locations[a].

	No-tillage sites			Tilled sites		
	1994	1995	1996	1994	1995	1996
Location	Barry Co.	Barry Co.	Shiaw. Co.	Kalam. Co.	Cass Co.	Kalam. Co.
Planting date	May 23	June 4	May 29	May 5	May 10	May 3
Application date	June 21	July 1	June 25	June 3	June 12	June 10
Corn height	20 cm	30 cm	30 cm	15 cm	23 cm	20 cm
Hemp dogbane height	51 cm	97cm	54 cm	41 cm	51 cm	61 cm
Hemp dogbane density	21 plants/m^2	16 plants/m^2	21 plants/m^2	19 plants/m^2	27 plants/m^2	19 plants/m^2

[a]Abbreviations: Shiaw. Co., Shiawassee County; Kalam. Co., Kalamazoo County.

Table 2. Control of treated hemp dogbane shoots 8 WAT at tilled and no-tillage sites.

		Hemp dogbane control					
		No-till			Tilled		
Treatment[a]	Rate	1994	1995	1996	1994[b]	1995	1996
	-------g/ha-------	%					
Nicosulfuron + NIS	18	24	22	28	38	62	33
Primisulfuron + NIS	20	8	30	30	28	74	25
Dicamba	280	27	59	91	36	87	15
2,4-D amine	560	53	98	98	31	93	83
Nicosulfuron + dicamba +NIS	18 + 280	78	99	99	59	95	57
Primisulfuron + dicamba + NIS	20 + 280	65	98	99	68	95	55
Nicosulfuron + 2,4-D amine +NIS	18 + 560	97	99	99	91	92	99
Primisulfuron + 2,4-D amine + NIS	20 + 560	98	99	99	88	95	99
Dicamba + 2,4-D amine	140 + 280	37	98	92	34	87	25
Dicamba	560	47	99	96	-	93	26
Dicamba + 2,4-D amine	280 + 280	32	97	96	-	89	38
Pro + prim + NIS	10+10	-	12	30	-	62	19
Pro+ prim+ dicamba + NIS	10 + 10 + 280	-	99	99	-	95	55
Pro+ prim + 2,4-D amine + NIS	10 + 10 + 560	-	99	99	-	95	99
Control		0	0	0	0	0	0
LSD(0.05)		14	16	8	16	13	10

[a]Abbreviations: NIS, nonionic surfactant; pro + prim, prosulfuron + primisulfuron.

[b]Last rating in 1994 was 42 DAT.

Table 3. Conditions at no-tillage sites in 1994, 1995, and 1996.

	Year		
Environmental factor	1994	1995	1996
Years in no-tillage	> 3 yr	> 3 yr	1 yr
GDD prior to treatment	431	899	700
Rainfall 6 weeks prior to treatment	8.1 cm	8.4 cm	20.8 cm

Table 4. Control of treated hemp dogbane shoots with various 2,4-D formulations.

		Hemp dogbane control					
		4 WAT			8 WAT		
Treatment[a]	Rate	1994	1995	1996	1994	1995	1996
	---g/ha---	%					
2,4-D amine A	560	35	58	67	50	93	80
2,4-D amine B	560	35	67	45	45	95	80
2,4-D ester A	560	90	83	89	99	96	99
2,4-D ester B	560	72	85	88	97	95	98
LSD(0.05)		10	7	27	15	NS	17

[a]2,4-D amine A, Weedar 64, dimethylamine salt of 2,4-D; 2,4-D amine B, Hi-Dep, dimethyl amine salt of 2,4-D plus diethanolamine salt of 2,4-D; 2,4-D ester A, Weedone LV4, butoxyethyl ester of 2,4-D; 2,4-D ester B, Weedone 638, butoxyethyl ester of 2,4-D plus parent acid of 2,4-D.

Figure 1. Boxplots of hemp dogbane control 6 WAT using combined data from six sites from 1994 to 1996. Black dots represent treatment means, boxes represent 50% of the data, and the whiskers represent 80% of the data.

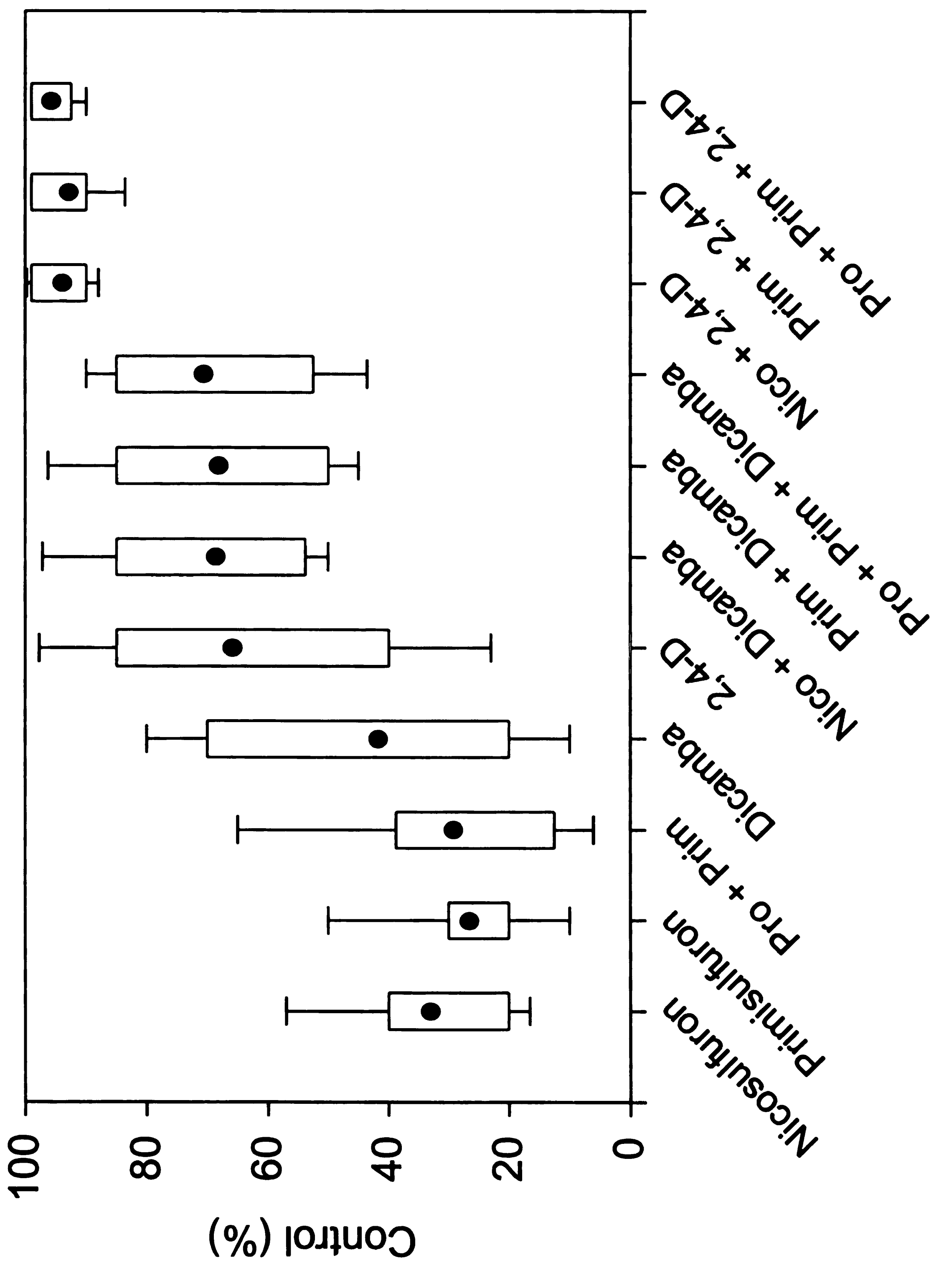
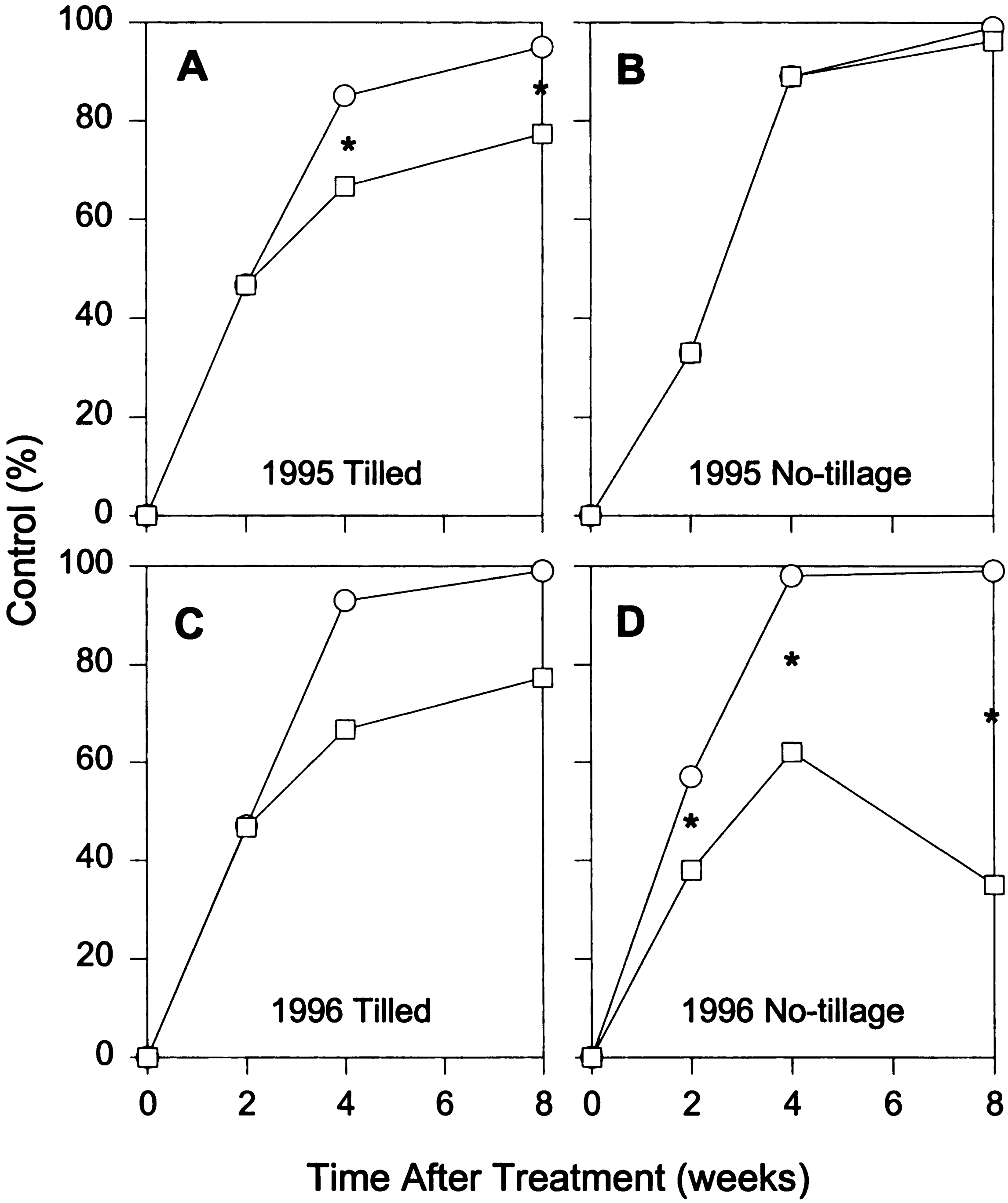

Control (%)
0
20
40
60
80
100
Nicosulfuron
Primisulfuron
Pro + Prim
Dicamba
2,4-D
Nico + Dicamba
Prim + Dicamba
Pro + Prim + Dicamba
Nico + 2,4-D
Prim + 2,4-D
Pro + Prim + 2,4-D

Figure 2. Evaluations of treated shoots (open circles) and treated plus new emerged shoots (open squares) for plots treated with a tank mixture of primisulfuron (20 g/ha), 2,4-D amine (560 g/ha), and NIS (0.25% v/v). Asterisks placed between the two ratings represent a significant difference between the ratings at the 0.10 confidence level. 1995 tilled (A), 1995 no-tillage (B), 1996 tilled (C), 1996 no-tillage (D).


100
80
60
40
20
0
Control (%)
A
1995 Tilled
B
1995 No-tillage
C
1996 Tilled
D
1996 No-tillage
0
2
4
6
8
Time After Treatment (weeks)